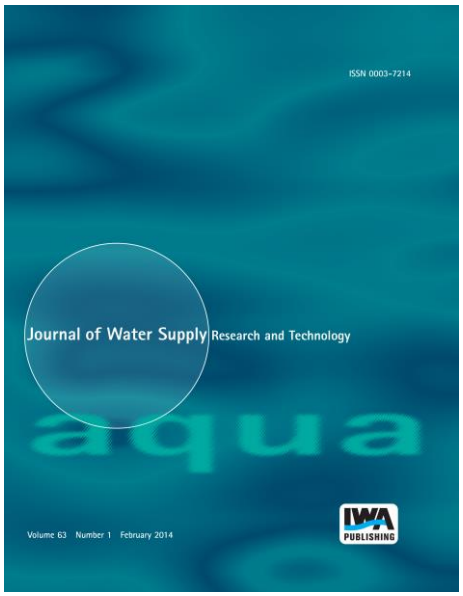

Bacterial biofilms formed in arsenic-containing water: bacterial community characterization

Silvia E. Rastelli, Blanca M. Rosales and Marisa R. Viera

ABSTRACT

We have analyzed the structure and diversity of bacterial biofilms formed on different water distribution materials in the absence and in the presence of 5 mg/L of arsenic(V). Three commercial metals (cast iron, zinc and copper) and polypropylene were used as substrata for biofilm formation. Biofilms were observed using scanning electron microscopy. Microbial ecology techniques (DNA amplification by polymerase chain reaction and denaturing gradient gel electrophoresis) were applied to study the bacterial community attached to each substratum. The presence of arsenic-tolerant bacteria was investigated. Microscopic observations showed that biofilm development was greater on those coupons exposed to the arsenic-containing water. The presence of arsenic also induced qualitative and quantitative changes in the planktonic bacterial community. Clustering analysis of the sessile community profiles showed that the nature of the substrata was a more important factor for the establishment of the community than the presence of arsenic in water. Culturable arsenic-tolerant bacteria were obtained from most of the biofilms. The genetic diversity of the community adapted to the presence of the contaminant was less affected by the high concentration of arsenic(V) in the culture, reflecting the presence of populations adapted to the contaminant.

Key words | arsenic, bacterial communities, biofilm, denaturing gradient gel electrophoresis, water

Silvia E. Rastelli
Blanca M. Rosales
Marisa R. Viera (corresponding author)
Centro de Investigación y Desarrollo de Pinturas (CIDEPINT; CICPBA- CCT La Plata CONICET),
Calle 52 entre 121 y 122,
1900 La Plata,
Argentina
E-mail: *marisa.rviera@gmail.com*

Silvia E. Rastelli
Facultad de Ciencias Naturales y Museo,
Universidad Nacional de La Plata,
Av.60 esq. 122,
1900 La Plata,
Argentina

Marisa R. Viera
Facultad de Ciencias Exactas,
Universidad Nacional de La Plata,
Calle 47 y 115,
1900 La Plata,
Argentina

INTRODUCTION

Microorganisms, including bacteria, present in natural and artificial aquatic environments, tend to attach to and grow on immersed surfaces, developing a biofilm (Dexter 2003). Biofilms formed on the internal surface of water distribution pipes may cause pipe plugging and act as a reservoir of microorganisms in drinking water distribution systems (WDSs) (Berry *et al.* 2006). Microbial influenced corrosion may damage the inner surface of pipelines making up a WDS. The pipe material, the presence of disinfectants, water flow velocity and many other variables influence biofilm characteristics (Lehtola *et al.* 2004). Also, microbes react when they are exposed to toxic environments: to avoid exposure to toxicity, microbes tend to form biofilms reducing the surface area in contact with the environment (Fang *et al.* 2002).

Arsenic is a toxic element that is widely distributed in the environment. Many communities around the world have high levels of arsenic in their waters because of contamination or the geology of the area. The Chaco Pampean Plain of central Argentina constitutes one of the largest regions of high arsenic groundwaters known; it covers around 1 million km^2 with arsenic levels from 0.6 to 4.8 mg/L (Farías *et al.* 2003; Carrera & Cirelli 2005). Despite arsenic's toxicity, a number of microorganisms are capable of growing in arsenic environments by incorporating it into their cells. Bacteria capable of removing arsenic from their surroundings could be very useful in a bioremediation process as an alternative or to supplement existing physicochemical methods of arsenic removal (Takeuchi *et al.* 2007).

doi: 10.2166/aqua.2015.137

Conventionally, many approaches to the investigation of biofilms concentrate on the disruption of the biofilm by scraping and evaluation of the growth of the recovered material on agar plates. However, this method has two main limitations. First, it results in the elimination of essential features such as the biofilm's complex structure and organization. Secondly, cultivation in a nutrient-rich medium is selective and does not involve the whole bacterial community: it has been estimated that 99.9% of bacterial cells in biofilms cannot be cultured on standard media (Wimpenny *et al.* 2000). The development of molecular techniques has allowed study of microbial communities by culture-independent methods. Among these techniques, denaturing gradient gel electrophoresis (DGGE) is commonly used for genetic fingerprint analysis of microbial community composition, diversity and dynamics (Green *et al.* 2009). DGGE has previously been used for studying the composition of biofilms in different drinking water treatment steps (Roeder *et al.* 2010).

As a part of a broad research project concerned with the effect of arsenic on water-distribution materials, in this paper we present our findings on the influence of the substratum and the presence of arsenic on the biofilm community. The presence of bacteria able to tolerate high arsenic concentration was investigated.

MATERIALS AND METHODS

Substrata and water source

Several materials commonly used in WDSs were selected for biofilm formation: commercial iron (Fe) (low carbon steel AISI 1005, used instead of cast iron), commercial zinc (Zn) (98% Zn, 1% copper, 1% titanium + magnesium, instead of galvanized steel), copper (Cu) (HIDRO-BRONZ®) and polypropylene (PP). To minimize laminar water-flux perturbation, metallic test samples were rolled and the PP thermocompressed up to 0.2 mm. Then they were cut to make 10×10 mm coupons. Metal surface finishing was done by manual abrasion up to 1,000 grade emery paper.

La Plata city drinking water was used as the microbial source and aqueous medium for colonization in all the experiments. The main physicochemical parameters of La Plata drinking water are presented in Table 1.

Water-circulation closed loops

Two laboratory model WDSs, each consisting of a 50 L polyvinyl chloride tank and a closed loop of PP tubes (inner diameter: 2.32 cm; length: 200 cm) filled with La Plata drinking water, were used (Figure 1). Water was pumped at a laminar flux with alternating 30/60 min work/stop periods to simulate network operating cycles. A removable transparent acrylic cell containing seven coupons of each substratum was placed in the loop. To study the influence of arsenic on the bacterial planktonic and sessile communities, 5 mg/L As(V) (in the form of $Na_2HAsO_4.7H_2O$, Biopack®) was added to one of the tanks. After a 45-day test period, the removable cells were extracted in sterile conditions. This procedure was repeated in ten independent runs.

Microscopic biofilm observations

Biofilm samples were prepared for scanning electron microscope (SEM) observations by fixation with glutaraldehyde (Sigma-Aldrich®) 2.5% in phosphate-buffered saline, dehydration with ethanol 20–100%, critical point drying and surface-conductive ultra-thin coating. A Philips SEM 505 coupled to an EDAX ultrathin window analyzer were used to obtain morphological observations and chemical analyses of the biofilms.

Table 1 | Main physicochemical parameters of La Plata drinking water

Parameter	
pH	6.5
Initial free Cl_2 (mg/L)	0.5–0.6
Ca^{2+} (mg/L)	17
Mg^{2+} (mg/L)	19
Na^{+} (mg/L)	75
K^{+} (mg/L)	5
Cl^{-} (mg/L)	130
SO_4^{2-} (mg/L)	105

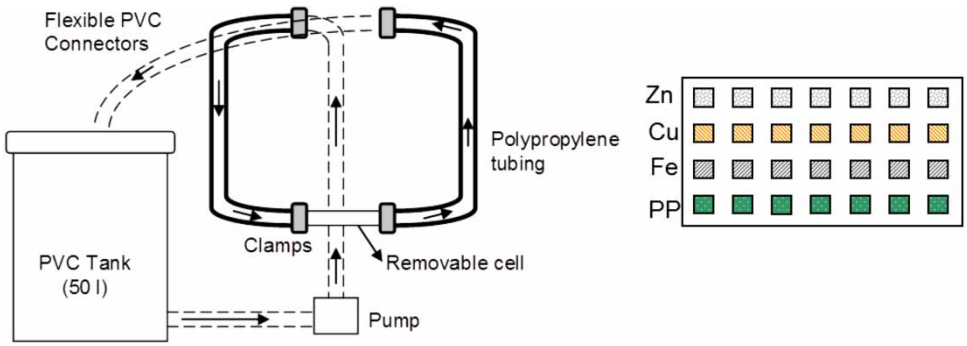

Figure 1 | Left: schematic diagram of the experimental set-up designed for water recirculation (arrows indicate the flow direction). Right: arrangement in the removable cell of the seven coupons of the four materials (Zn, Cu, Fe and PP) used for biofilm formation.

Enumeration of heterotrophic and As-tolerant sessile bacteria

Three of the seven coupons of each material removed from the cells were used to obtain a sessile heterotrophic plate count (HPC) in triplicate. The attached material (biofilm + extracellular polymeric substance + corrosion products) was scraped with a sterile scalpel. The material obtained from each coupon was suspended in 1 mL sterile physiological solution, serially diluted, and 0.1 mL aliquots were seeded on nutrient agar (plurypeptone 5 g/L; beef extract 3 g/L; NaCl 8 g/L; agar 15 g/L) (Britania®) and incubated at 25 °C for 72 h. The As-tolerant culturable sessile bacteria were obtained by culturing 0.5 mL in nutrient broth (Biokar Diagnostics) with 50–1,000 mg/L As(V). The estimation of the tolerant bacteria was done by the dilution to extinction method in nutrient broth.

Bacterial community characterization

To analyze the microbial community, the total DNA of the planktonic and sessile bacteria was extracted. Planktonic DNA was obtained by filtering 1 L water from each tank through a 0.22 μm sterile cellulose acetate membrane (Sartorius) at the end of the experiment. For sessile DNA extraction, the remaining material scraped from the three coupons used for enumeration, plus the material from a fourth coupon, were combined, centrifuged at 13,000 *g* for 15 min and the supernatant discarded. Total and culturable sessile and total planktonic DNA was extracted using an E.Z.N.A. Soil DNA kit (Omega Bio-tek) following the manufacturer's instructions. The 16S rRNA gene sequences were amplified from the DNA extracted from the water samples and the biofilms by polymerase chain reaction (PCR) in a Mastercycler® ep (Eppendorf) machine using the universal primers for 341F with a GC clamp (Eurofins MWG Operon) and 907R (Eurofins MWG Operon), as described by Green *et al.* (2009). The amplification conditions were as follows: an initial denaturation step at 94 °C for 4 min, followed by 10 cycles of 94 °C for 30 sec, 62 °C for 45 sec, 72 °C for 60 sec, then 25 cycles of 94 °C for 30 sec, 57 °C for 45 sec, 72 °C for 60 sec and a final extension at 72 °C for 10 min. Negative controls (without DNA) were run in all the amplifications and the presence of PCR product was confirmed by 1.2% w/v agarose gel electrophoresis and Sybr® Gold (Invitrogen) staining.

DGGE was performed using DGGE 2401 apparatus (CBS Scientific) in a 6% (w/v) polyacrylamide gel with a 30–70% denaturant gradient (100% denaturant is 7 M urea and 40% v/v formamide) loaded with the PCR products (10–15 μL). Electrophoresis was performed in TAE buffer for 16 h at 60 °C, at 100 V. The gels were stained with Sybr® Gold for 40 min and observed and photographed in a UV transillumination Universal Hood II (Bio Rad). For statistical evaluation, the gels were analyzed using the Gel Compare II software (Applied Maths). The band-based Dice coefficient was used to calculate the similarity matrix with a position tolerance of 1%. The unweighted pair group method with arithmetic mean (UPGMA) was applied for clustering.

Identification of bacteria

From the nutrient agar plates used for HPC enumeration, isolated colonies were picked and inoculated on a new

plate and incubated at 25 °C for 48 h. DNA was extracted by suspending, with the aid of an inoculating loop, a colony in an Eppendorf tube with 1 mL of sterile distilled water and boiling for 10 min. Then, the Eppendorf was centrifuged at 13,000 *g* for 5 min and the supernatant was transferred to a new Eppendorf tube. From the DNA obtained, PCR amplification of almost the whole 16S rRNA gene sequence was carried out in a Mastercycler® ep (Eppendorf) using the primers 27F and 1541R (Eurofins MWG Operon) with the following conditions: an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of 95 °C for 30 sec, 48 °C for 60 sec and finally an extension at 72 °C for 10 min. Negative controls were run in all the amplifications and the presence of PCR product was confirmed by 1.2% w/v agarose gel electrophoresis and Sybr® Gold staining. The PCR products were purified using the commercial kit GeneClean®III (MP Biomedicals) and sequenced by MACROGEN (Korea) using the universal primers for the 16S rRNA gene 27F and 1492R. Sequence data were compared for initial identification, with the closest relatives represented by the retrieved sequences obtained from homology searches using the Blast algorithm at the NCBI (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS AND DISCUSSION

Total and arsenic-tolerant heterotrophic sessile bacteria enumeration

HPCs were obtained from the four materials assayed in the presence and absence of As(V) in the water (Table 2). Biofilms developed on Fe and Zn gave the highest counts, while HPC in biofilms formed on Cu and PP were up to two orders of magnitude lower, in agreement with the observations made by other authors who found that the amount of bacteria attached to Fe or steel was higher than that on plastic or Cu pipes (Yu *et al.* 2010; Wang *et al.* 2012). Slightly higher values were found for biofilms developed in the As-containing water except in the case of biofilms formed on Cu. In the latter case, the low counts in both systems could be related to the release of toxic Cu ions from the surface (Yu *et al.* 2010). The higher number of attached bacteria in the presence of As correlated with the analysis of the confocal laser microscope images performed (Rastelli *et al.* 2015), which indicated that the surface coverage was significantly higher on those coupons exposed to the As-containing water, except for biofilms formed on Cu. This observation agrees with those by other authors (Fang *et al.* 2002) regarding the effect of the presence of toxic compounds in liquid media as promoting the development of the sessile over the planktonic form.

Table 2 | HPC (colony-forming units/cm^2) of the biofilms formed on the different substrata assayed (Fe, Zn, Cu and PP) in both circuits (with or without As(V))

	Without As	With As
Fe	$3.35\ 10^4$	$6.29\ 10^4$
Zn	$5.00\ 10^4$	$3.27\ 10^5$
Cu	$1.06\ 10^4$	$1.78\ 10^3$
PP	$4.31\ 10^3$	$6.60\ 10^3$

The numbers represent the average of ten independent experiments, with three replicates per experiment.

Microscopic characterization of biofilm

Details of the biofilms were observed with SEM (Figure 2). All the materials showed the presence of bacteria in contact with the substratum. A single phase biofilm was noticed on Cu and PP, while a more complex structure was observed on Fe and Zn. The energy-dispersive X-ray spectroscopy (EDS) of samples exposed to As revealed its presence only in areas covered by biofilm, in a percentage proportional to the amount of deposit accumulated. This result suggests that As interacted with the biological components of the deposits. It has been demonstrated that bacteria can participate in As uptake mainly by two mechanisms: adsorption and active incorporation into the cell (Takeuchi *et al.* 2007).

Planktonic and sessile bacterial communities

Direct PCR-DGGE based on the 16S rRNA gene of the planktonic and sessile DNA allowed a description of the structure of the microbial communities present in the water and in the biofilms, respectively. In our analysis, the number, position and intensity of the bands were taken as an indication of the number and relative abundance of the dominant species present in each sample. As

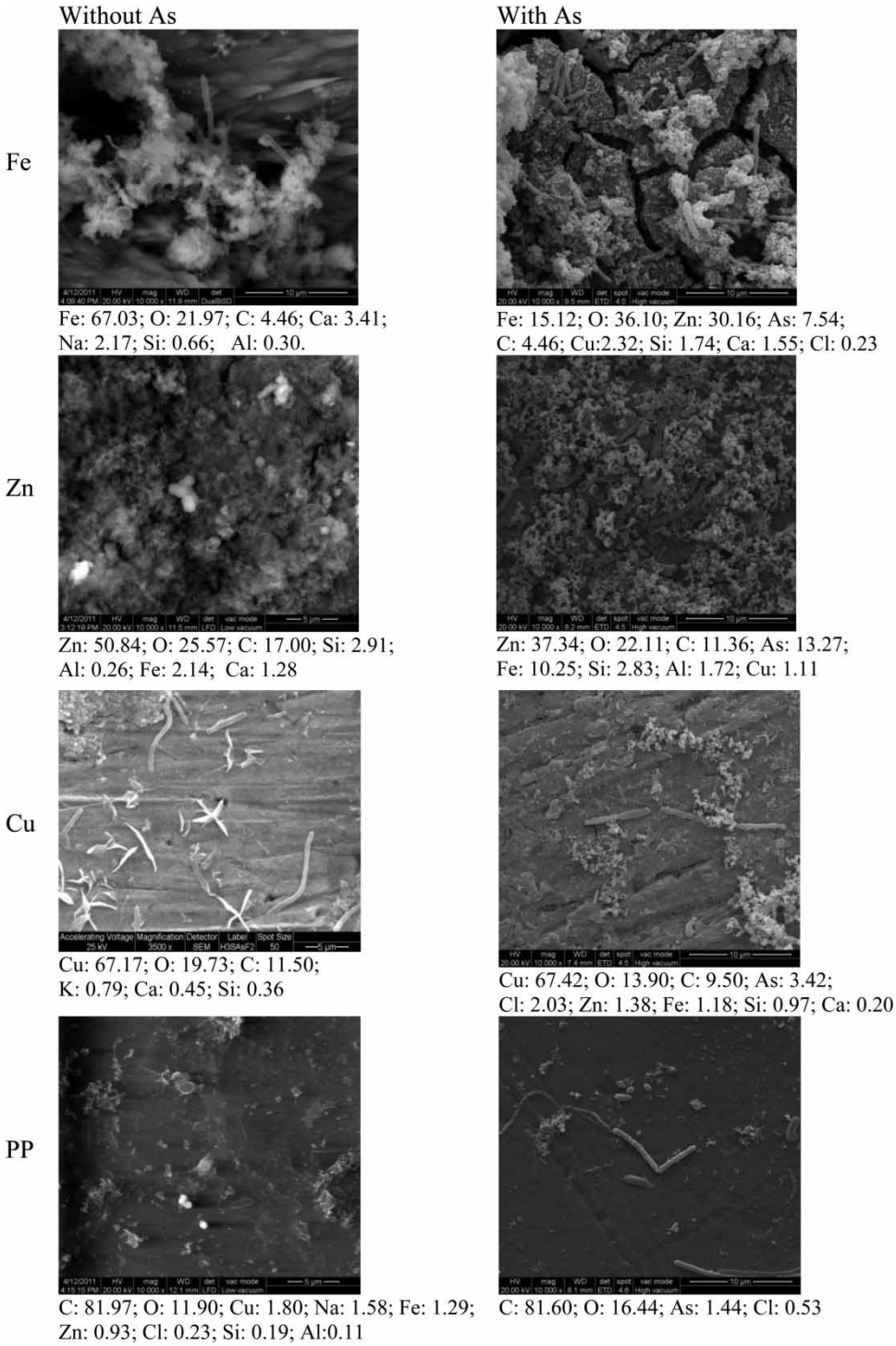

Figure 2 | SEM-EDS analysis of the biofilms developed on Fe, Zn Cu and PP coupons exposed to water without or with 5 mg/L As(V) (EDS values of the main elements are expressed in percentages).

with other methods, DGGE only provides an indication but not an absolute measure of the degree of biodiversity in a bacterial community (Rosch *et al.* 2006). Figure 3 illustrates the DGGE band patterns corresponding to the planktonic

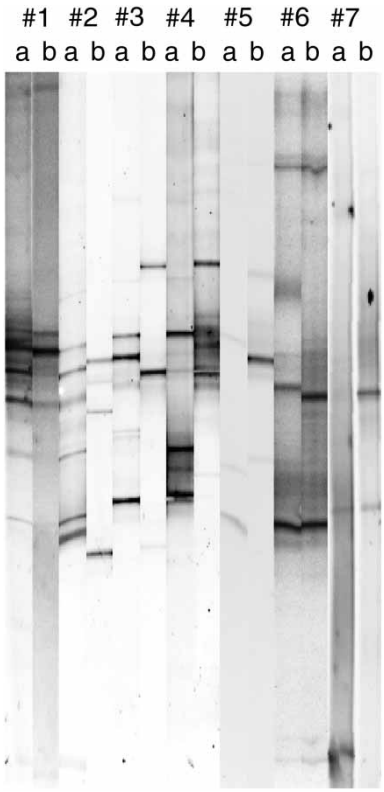

Figure 3 | DGGE profiles of amplified bacterial 16S rDNA fragments from the planktonic communities in: (a) the circuit without As(V); and (b) with 5 mg/L As(V). #1 to #7 indicates the experiment number. Gradient of urea and formamide ranged from 30 to 70%.

communities presented in both circuits in seven independent experiments. It can be seen that each sample produced a distinctive DGGE profile with a different number of bands of diverse intensity, which was different from the profiles generated by any other sample. Besides, the diversity of the bacterial community was very variable among experiments. In several experiments, a relatively high number of populations was found (experiments 1a, 5a and 5b with 13, 15 and 12 identifiable bands, respectively), while other experiments exhibited very few bands (experiments 3b, 6a, 7a and 7b with 5, 4, 3 and 3 bands respectively). At the beginning of each experiment, both circuits were filled with the same water source, then As(V) was added to one of them, thus the same populations were originally present in both circuits. The different profiles in both circuits at the end of each experiment indicated that the bacterial community was affected by the presence of As in the water. As shown in Figure 3, the presence of As induced qualitative (band position) and quantitative (band intensity) changes in the microbial community. Studies of the effects of pollutants on the overall genetic structure and diversity of the whole bacterial community found that the exposure to heavy metals alters these characteristics (Li *et al.* 2006). In general, a decrease in the genetic diversity as a result of the presence of a toxin was observed. However, it was found that the presence of mercury in soil induced an initial decrease in the bacterial genetic diversity followed by a gradual increase in the diversity with the appearance of new dominating bands reflecting an adaptation of the community to the pollutant (Rasmussen & Sörensen 2001).

Profiles of the sessile communities formed on each material were also obtained. Due to the low number of bacteria in the biofilms, DNA extraction was unsuccessful for several biofilms, particularly on PP and Cu. Only experiments 1 and 7 yielded measurable amounts of DNA (between 39 and 300 ng/mL) from biofilms formed on all the substrata. The profiles of the bacterial sessile communities developed on each substratum in those experiments are shown in Figure 4(a). In experiment 1 only, one band could be detected in all the biofilms, indicating the existence of a population able to attach to all the substrata. Consistent with the low number of bands obtained for the planktonic community, biofilms from experiment 7 exhibited low diversity. The comparison between the community profile on each substrata in the absence or in the presence of As(V) did not show any significant difference (with the exception of the biofilms formed on Zn in experiment 1). The clustering analysis (Figure 4(b)) shows a clear trend for biofilms formed on a particular material to cluster together regardless of the presence of As in the liquid phase. Exceptions to this behavior were the biofilms developed on Zn and on PP in both circuits in experiment 1. Biofilms formed on Fe (experiment 1) and PP and Cu (in experiment 7) in the presence or in the absence of As had a similarity of 100%. These findings indicated that the nature of the substrata was a more important factor for the establishment of the sessile community than the presence of As. It has been reported that surface properties affected biofilm community composition (Iasur-Kruh *et al.* 2010). Certain materials can release different compounds, such as phosphorus from plastics, iron or copper from steel or copper pipes, that can

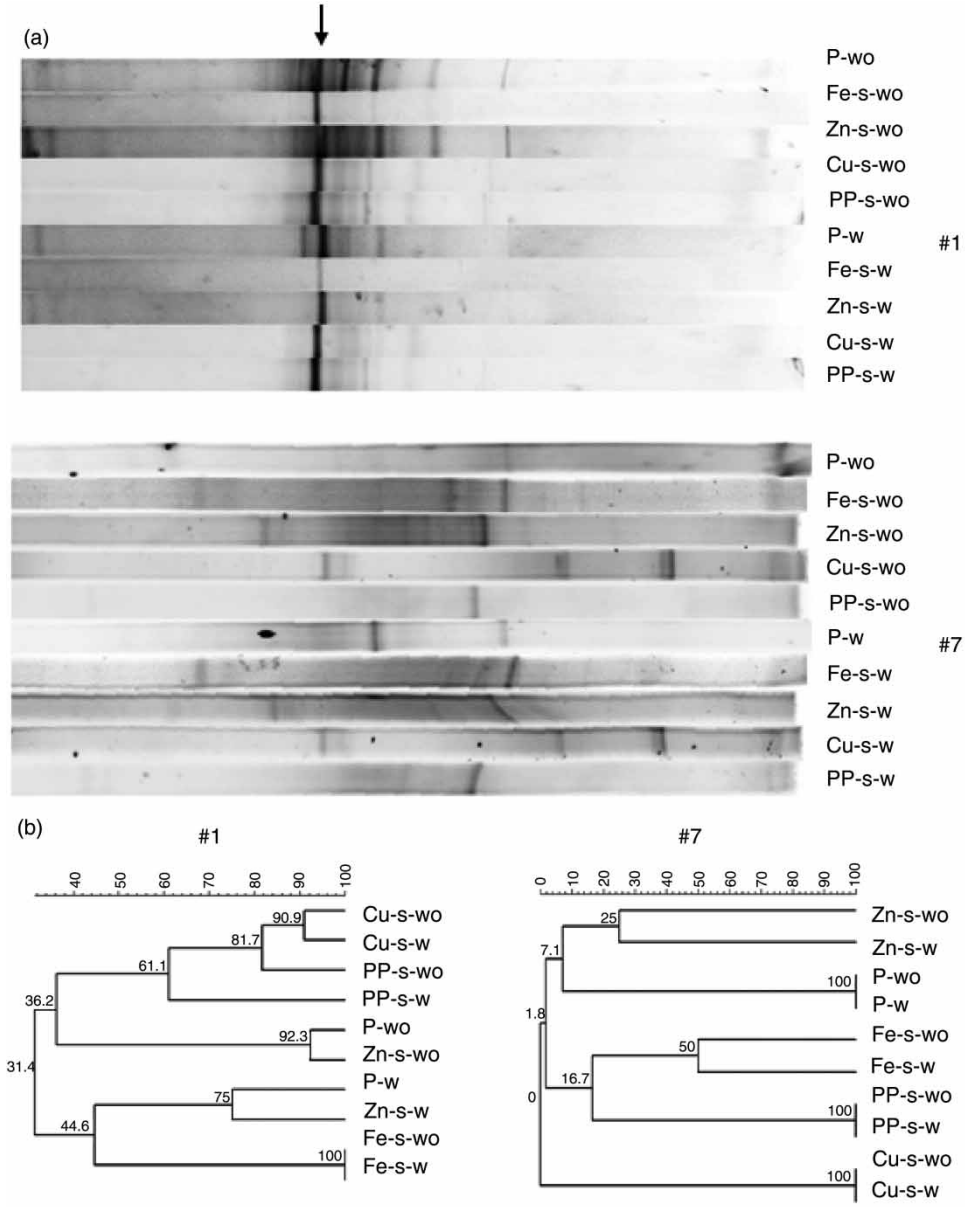

Figure 4 | (a) DGGE profiles of amplified bacterial 16S rDNA fragments from the sessile communities developed on Fe, Zn, Cu and PP; in experiments 1 and 7 in both circuits (without As and with 5 mg/L As(V)). The corresponding planktonic profiles are also included. wo: without As(V); w: with As(V); s: sessile community; P: planktonic community. Gradient of the urea and formamide ranged from 30 to 70%. (b) The corresponding cluster analysis.

influence biofilm development (Lehtola *et al.* 2004). In our case, the community profile developed on different materials differed, as well as the magnitude of the attack produced on the substrata (Rastelli *et al.* 2015). A high similarity amongst the established community on the materials less susceptible to bacterial attack (PP and Cu) was observed. These results suggested that a relationship could exist amongst the established community and its deterioration effect on the substratum.

Identification of bacteria

Among the colonies obtained on nutrient agar plates seeded with the material scraped from the biofilms, 34 isolates

could be identified through the amplification of the near full-length 16S RNA gene sequences; 15 species (with a similarity higher than 94%) from eight genera were present. Only microorganisms belonging to the class Bacilli (representing 44% of identified colonies) and Alpha-Proteobacteria (35% of the identified colonies) were found on all the substrata in the presence and in the absence of As(V). The class Actinobacteria (6% of the identified colonies) was found only on Cu and PP coupons exposed on the circuit with As(V). Members of the Beta-Proteobacteria (12%) were isolated from biofilms developed on Zn and PP in the presence of As(V), and Gamma-Proteobacteria (3%) were found only on Zn coupons in the absence of As (V). The percentages of abundance and distribution of the bacterial classes in the biofilms formed on the four substrata exposed in both systems (with and without As(V)) are shown in Figure 5. The taxonomic identity of each strain according to the comparison with the NCBI Genbank Database is presented in Table 3 with their accession numbers.

Arsenic-tolerant bacteria

Biofilms scraped from all the coupons were cultured in different As(V) concentrations. Culturable As-tolerant bacteria were obtained from all the biofilms except Cu-biofilms cultured in the highest As(V) concentrations. The number of arsenic-tolerant bacteria obtained by the dilution to extinction method diminished as the As(V) concentration increased, and was higher in biofilms from the As-containing circuit at the highest As concentrations (Table 4). Arsenic-resistant bacteria have been isolated from arsenic rich environments such as arsenic-contaminated soils (Turpeinen *et al.* 2004). However, other studies revealed large proportions of arsenic-resistant bacteria in arsenic-free environments (Jackson *et al.* 2005). In our case, we found arsenic-resistant bacteria in both systems, with a higher number in the biofilms formed in the arsenic-containing water, indicating a degree of adaptation.

To further study the adaptation of the bacterial populations to the presence of As, the DGGE band profiles of biofilms formed on Zn from both circuits, cultured in the presence of 500 and 1,000 mg/L As(V), were compared (Figure 6). It can be seen that even at such high As concentrations, the number and intensity of the bands in the lanes corresponding to culturable bacteria were higher than those obtained in the original biofilm. This is related to the increase in the number of copies of bacterial DNA due to culturing. The diversity of the culturable heterotrophic fraction of the bacterial community has been analyzed in several papers. Whereas some authors found that the presence of a contaminant decreases the culturable bacterial diversity, other authors agree that the contaminant increases it (Rasmussen & Sörensen 2001; Dell'Amico *et al.* 2008). In our case, the genetic diversity of the community adapted to the contaminant was less affected by the high concentration of As(V), indicating the presence of some bacterial populations already adapted to the contaminant.

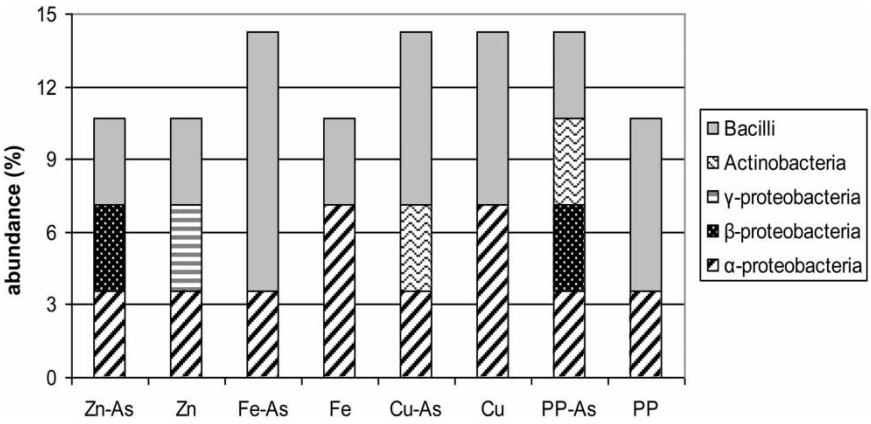

Figure 5 | Abundance (in percentage) of the different bacterial classes found in the biofilms formed on all the materials assayed (Fe, Zn, Cu and PP) in the absence or the presence of 5 mg/L As(V) in the water.

Table 3 | Identification of the bacterial isolates from different biofilms

Isolate number (source sample)	GenBank accession number	Similarity (%)	Closest relative in GenBank
Bacilli			
78 (Fe–As)	KM349188	96	*Bacillus licheniformis* strain SeaH-As1w
90 (PP–As)	KM349189	98	*Bacillus* sp. AsK 15
165 (PP)	KM349191	100	*Bacillus cereus* strain DZ4
170 (Cu)	KM349192	99	*Bacillus megaterium* strain MBFF6
178 (Fe–As)	KM349194	98	*Paenibacillus* sp. Q8
188 (Cu–As)	KM349197	99	*Bacillus* sp. SXB
190 (Cu–As)	KM349198	99	*Bacillus* sp. SXB
194 (Fe)	KM349200	99	*Bacillus circulans* strain WZ12
197 (Zn)	KM349201	99	*Bacillus circulans* strain WZ12
199 (Cu)	KM349203	98	*Bacillus circulans* strain WZ12
202 (PP)	KM349204	99	*Bacillus circulans* strain WZ12
206 (Fe–As)	KM349205	100	*Bacillus* sp. A103–77
210 (Zn–As)	KM349207	100	*Bacillus* sp. A103–77
212 (Cu–As)	KM349208	99	*Bacillus* sp. A103–77
214 (PP–As)	KM349185	99	*Bacillus* sp. A103–77
α-Proteobacteria			
192 (Fe)	KM349199	99	*Sphingomonas* sp. XJ-3
198 (Cu)	KM349202	99	*Sphingomonas* sp. XJ-3
218 (PP)	KM349210	99	*Brevundimonas* sp. NBRC 101024
221 (Cu)	KM349211	97	*Brevundimonas* sp. NBRC 101024
222 (Cu)	KM349212	99	*Brevundimonas* sp. NBRC 101024
224 (Fe)	KM349213	99	*Brevundimonas* sp. NBRC 101024
227 (Zn)	KM349214	99	*Brevundimonas* sp. NBRC 101024
230 (PP–As)	KM349215	87	*Brevundimonas* sp. OS16
232 (Cu–As)	KM349216	97	*Brevundimonas* sp. OS16
235 (Fe–As)	KM349217	96	*Brevundimonas* sp. OS16
238 (Zn–As)	KM349218	96	*Brevundimonas* sp. OS16
239 (Zn–As)	KM349219	96	*Brevundimonas* sp. OS16
β-Proteobacteria			
91 (PP–As)	KM349190	99	*Delftia* sp. TS33
183 (PP–As)	KM349195	99	*Delftia* sp. TS33
184 (PP–As)	KM349196	99	*Delftia* sp. TS33
209 (Zn–As)	KM349206	100	*Delftia* sp. TS33
γ-Proteobacteria			
175 (Zn)	KM349193	97	*Acinetobacter lwoffii* strain Cl-01
Actinobacteria			
69 (Cu–As)	KM349187	96	*Kokuria* sp. TS13
213 (PP–As)	KM349209	94	*Janibacter* sp. TS20

Table 4 | Enumeration of As-tolerant bacteria, by dilution to extinction, in the biofilms formed on Fe, Zn, Cu and PP in circuits (with and without 5 mg/L As(V)), cultured at different As(V) concentrations

	As(V) concentration (mg/L)					
Sample	**50**	**100**	**200**	**300**	**500**	**1,000**
Zn (A)	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6	10^4–10^5
Zn	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6	10^4–10^5	10^3–10^4
Fe (A)	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6
Fe	10^5–10^6	10^5–10^6	10^5–10^6	10^5–10^6	10^3–10^4	10^4–10^5
Cu (A)	10^4–10^5	10^5–10^6	10^5–10^6	10^5–10^6	ng	ng
Cu	10^4–10^5	10^5–10^6	10^5–10^6	10^5–10^6	ng	ng
PP (A)	10^4–10^5	10^5–10^6	10^5–10^6	10^5–10^6	10^2–10^3	10^2–10^3
PP	10^4–10^5	10^5–10^6	10^5–10^6	10^5–10^6	nd	nd

A: indicates biofilm from the As-containing circuit; ng: no growth detected; nd: no data.

Figure 6 | DGGE fingerprint gel of 16S rDNA of sessile communities developed on Zn with and without As(V) and the planktonic culturable communities obtained from them, when the biofilms were cultured in the presence of 500 or 1,000 mg/L As(V). The corresponding planktonic community is also included in the analysis. Gradient of urea and formamide ranged from 30 to 70%. P: planktonic; s: sessile: c: culturable; w: from system with 5 mg/L As(V); wo: from system without As(V); 500 or 1,000 indicates the As(V) concentration (in mg/L) in the cultures.

The presence of bacteria able to tolerate high As concentrations could be relevant for the implementation of a bioremediation process based on As uptake by microorganisms.

CONCLUSIONS

Bacteria present in water were able to grow and developed biofilms on all the materials tested. The number of bacteria attached to Fe and Zn were higher than those found on Cu and PP. Slightly higher values were found for biofilms developed in the As-containing circuit, except in the case of biofilms formed on copper. The observation that As was detected only in areas covered by biofilm suggests that bacteria in the biofilm were involved in As uptake.

The presence of As(V) in the water induced qualitative and quantitative changes in the planktonic bacterial community. However, for the establishment of the sessile community, the nature of the substrata was a more important factor than the presence of As.

Culturable As-tolerant bacteria were obtained from most of the biofilms originating in both circuits. The genetic diversity of the community adapted to the presence of the contaminant was less affected by the high concentration of As(V) in culture, reflecting the presence of populations adapted to the contaminant.

ACKNOWLEDGEMENT

Financial support for this work from the governmental Agencia Nacional de Promoción Científico-Tecnológica, PICT 38380, is gratefully acknowledged.

REFERENCES

Berry, D., Xi, C. & Raskin, L. 2006 Microbial ecology of drinking water distribution systems. *Curr. Opin. Biotech.* **17**, 297–302.

Dell'Amico, E., Mazzocchi, M., Cavalca, L., Allievi, L. & Andreoni, V. 2008 Assessment of bacterial community structure in a long-term copper-polluted ex vineyard soil. *Microbiol. Res.* **163**, 671–683.

Dexter, S. C. 2003 Microbiologically influenced corrosion. In: *ASM Handbook. Corrosion: Fundamentals, Testing and Protection* (S. D. Cramer & B. S. Covino Jr, eds). ASM International, Ohio, USA, pp. 398–416.

Fang, H., Xu, L.-C. & Chan, K.-Y. 2002 Effects of toxic metals and chemicals on biofilm and biocorrosion. *Water Res*. **36**, 4709–4716.

Farías, S., Casa, V., Vázquez, C., Ferpozzi, L., Pucci, G. & Cohen, I. 2003 Natural contamination with arsenic and other trace elements in ground waters of Argentine Pampean Plain. *Sci. Total Environ*. **309**, 187–199.

Green, S., Leigh, M. & Neufeld, J. 2009 Denaturing gradient gel electrophoresis (DGGE) for microbial community analysis. In: *Microbiology of Hydrocarbons, Oils, Lipids, and Derived Compounds* (K. N. Timmis, ed.). Springer, Heidelberg, Germany, pp. 4137–4158.

Iasur-Kruh, L., Hadar, Y., Milstein, D., Gasith, A. & Minz, D. 2010 Microbial population and activity in wetland microcosms constructed for improving treated municipal wastewater. *Microbial Ecol*. **59**, 700–709.

Jackson, C., Dugas, S. & Harrison, K. 2005 Enumeration and characterization of arsenate-resistant bacteria in arsenic free soils. *Soil Biol & Biochem*. **37**, 2319–2322.

Lehtola, M., Miettinen, I., Keinänen, M., Kekki, T., Laine, O., Hirvonen, A., Vartiainen, T. & Martikainen, P. 2004 Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Res*. **38**, 3769–3779.

Li, Z., Xu, J., Tang, C., Wu, J., Muhammad, A. & Wang, H. 2006 Application of 16S rDNA-PCR amplification and DGGE fingerprinting for detection of shift in microbial community diversity in Cu-, Zn-, and Cd-contaminated paddy soils. *Chemosphere* **62**, 1374–1380.

Pérez Carrera, A. & Fernández Cirelli, A. 2005 Arsenic concentration in water and bovine milk in Cordoba, Argentina. Preliminary results. *J. Dairy Res*. **72**, 122–124.

Rasmussen, L. & Sörensen, S. 2001 Effects of mercury contamination on the culturable heterotrophic, functional and genetic diversity of the bacterial community in soil. *FEMS Microbiol. Ecol*. **36**, 1–9.

Rastelli, S., Rosales, B., Viera, M. & Elsner, C. 2015 Bacterial biofilms formed in arsenic-containing water: biodeterioration of water network materials. *J. Water Supply Res. Technol. AQUA* **64** (6), 738–748.

Roeder, R., Lenz, J., Tarne, P., Gebel, J., Exner, M. & Szewzyk, U. 2010 Long-term effects of disinfectants on the community composition of drinking water biofilms. *Int. J. Hyg. Envir Heal*. **213**, 183–189.

Rosch, C., Eilmus, S. & Bothe, H. 2006 Approaches to assess the biodiversity of bacteria in natural habitats. *Biochem. Soc. T*. **34**, 169–173.

Takeuchi, M., Kawahata, H., Gupta, L., Kita, N., Morishita, Y., Ono, Y. & Komai, T. 2007 Arsenic resistance and removal was evaluated in nine bacterial strains of marine and non-marine origins. *J. Biotechnol*. **127**, 434–442.

Turpeinen, R., Kairesalo, T. & Häggblom, M. 2004 Microbial community structure and activity in arsenic-, chromium- and copper-contaminated soils. *FEMS Microbiol. Ecol*. **47**, 39–50.

Wang, H., Hu, X. & Hu, C. 2012 Effect of chlorine and pipe material on biofilm development and structure in a reclaimed water distribution system. *Water Sci. Technol: Water Supply* **12** (3), 362–371.

Wimpenny, J., Manz, W. & Szewzyk, U. 2000 Heterogeneity in biofilms. *FEMS Microbiol. Rev*. **24**, 661–671.

Yu, J., Kim, D. & Lee, T. 2010 Microbial diversity in biofilms on water distribution pipes of different materials. *Water Sci. Technol*. **61** (1), 163–171.

First received 29 September 2014; accepted in revised form 22 July 2015. Available online 4 September 2015