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RESEARCH ARTICLE

# Possible use of *Serratia marcescens* in toxic metal biosorption (removal)

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**Abstract**
*Background, aim and scope* Metal pollution is a serious problem for environmental safety and programmes of monitoring and bioremediation are needed. Among the processes of bioremediation, the use of microbes to remove and degrade contaminants is considered a biotechnological approach to clean up polluted environments.
*Aim* The aim of this study was to evaluate the ability of *Serratia marcescens* in Pb, Cd and Cr removal and the potential use of these bacteria in toxic metal bioremediation from polluted environments.
*Methods* A short-term study (120 min) was carried out to study the bacterial growth in the presence of sub-inhibitory concentrations of each metal analysed and the kinetics of metal biosorption in *S. marcescens* strain. In addition, metal influence on the biosynthesis of the red pigment 'prodigiosina' by *S. marcescens* was monitored.
*Results* The results obtained in this study show metals biosorption by *S. marcescens* (range: 0.0133–0.213 μg/g for Pb; 0.097–0.1853 μg/g for Cd; and 0.105–0.176 μg/g for Cr) and confirm the possible use of this bacterium to realize bioremediation processes, especially for Pb removal, and as a bioindicator of metal pollution.

**Keywords** Metal pollution · *Serratia marcescens* · Prodigiosin · Biosorption · Bioindicator

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## 1 Introduction

Environmental metal pollution is increasing as result of many anthropic activities (industrialization, fuel combustion, smelting processes, roadway traffic) and can be a serious threat for ecosystems and the safety of living organisms. To evaluate toxic metal pollution, programmes of environmental monitoring and systems of metal bioremediation and removal have been developed (Erdei et al., 2005; Le Duc and Terry 2005; Pulford and Watson, 2003; Lovley and Coates, 1997 Benguella and Benaissa 2002; Saeed and Iqbal 2003; Deng et al. 2007; Chen et al. 2009). These conventional physicochemical methods are economically expensive and have disadvantages like incomplete metal removal, higher reagent, energy requirements and generation of toxic sludge; new technologies are required to mitigate heavy metal concentrations to environmentally acceptable levels. To realize the bioremediation systems, the role of soil/sediment

microbial community in metal scavenging and release has been studied (Rani et al., 2010; Sobolev and Begonia 2008). Even though metals in free ionic forms are a significant toxic factor to biota in the environment, studies on bacterial diversity in heavy metal contaminated sites have demonstrated a high diversity of microorganisms (Filali et al. 2000; Ellis et al. 2003; Konstantinidis et al. 2003; Chien et al. 2008), useful for environmental monitoring (Demoling and Baath 2008). Microorganisms, ubiquitous and essential for the global biogeochemical cycling of elements (Haferburg and Kothe 2007), due to high surface and contact area, can easily interact with metals, adapt themselves physiologically to survive and remain active in condition of environmental stresses (Wang et al. 2010). Bacteria can be specific to one or a few metals (Mejare and Bulow 2001) and bind high concentrations of heavy metals (Kang et al. 2007; Kretschmer et al. 2004; Yee et al. 2004; Bae et al. 2000) according to a variety of mechanisms: They can potentially accumulate metals either by a metabolism-independent (passive) or a metabolism-dependent (active) process and can remove heavy metals through bioaccumulation or biosorption. In the bioaccumulation process, metals are transported from the outside of the microbial cell through the cellular membrane into the cell cytoplasm, where the metal is sequestered. Metals adsorption is determined by the sorptivity of the cell envelope and influenced by differences in the cell wall construction of Gram-positive and Gram-negative bacteria (Jiang et al., 2004); by the presence of phosphoryl groups, lipopolysaccharides, carboxylic groups, teichoic and teichuronic acids; and by parameters such as toxicity, composition and total content of metals (Haferburg and Kothe 2007).

The use of microbes to reduce, degrade or immobilize hazardous contaminants and clean up polluted environments results to be useful in bioremediation technology (Erdei et al. 2005; Le Duc and Terry 2005; Benguella and Benaissa 2002; Saeed and Iqbal 2003; Deng et al. 2007; Chen et al. 2009), as demonstrated by metal bioremediation processes of industrial wastes (Srivastava et al. 2008) and polluted effluents (Shukla et al. 2009; Malekzadeh et al. 2002) using bacteria such as *Pseudomonas* spp., *Vallisneria spiralis*, etc.

*Serratia marcescens* is a Gram-negative bacterium of the Enterobacteria family with a short, spherical and stick structure, size of 0.5 × 0.5/1 μm, found in various ecological niches, including soil, water, air, plants, animals and food in general. It is a pigmented bacterium which is a producer of 'prodigiosina', a red pigment with antiprotozoal, antifungal and antibacterial activities, a secondary metabolite obtained by the condensation of 4-methossi-2,2′ bipyrrolo-5 carboxi-aldeid and 2-metal-3-amil-pyrrol in the presence of a suitable ratio of carbon and nitrogen sources (Pizzimenti et al. 1983). Prodigiosin biosynthesis depends on bacterium growth conditions, is affected significantly by factors such as temperature, ions, presence of amino acids, and is considered as a protective mechanism in unfavourable conditions for bacterial growth (Hejazi and Falkiner 1997). *S. marcescens* can be considered a bioindicator of environmental pollution for the bioremediation of soil by organophosphorus insecticide diazinon (Cycon et al. 2009), degradation of dichlorodiphenyltrichloroethane (Bidlan and Manonmani 2002), diesel degradation and corrosion (Rajasekar et al. 2007), biotransformation of chlorpyrifos (Lakshmi et al. 2008) and pentachlorophenol (Singh et al. 2008). Some studies have evaluated the use of this bacterium for the degradation of chromium (Shukor et al. 2008; Campos et al. 2005), molybdenum (Yunus et al. 2009; Shukor et al. 2008) and nickel (Kannan and Ramteke 2002).

The purpose of this investigation was to evaluate the rule of bacterial strains of *S. marcescens* in the removal of toxic metals Pb, Cd and Cr and the potential use of this bacteria as bioindicators in the metal bioremediation processes. For this reason, Pb, Cd and Cr kinetics of accumulation by *S. marcescens* was monitored, verifying the metals' influence on bacterial growth and biosynthesis of the red pigment 'prodigiosin'.

## 2 Materials and methods

### 2.1 Reagents and chemicals

$K_2HPO_4$, $KH_2PO_2$, $NH_4Cl$, $FeCl_3$, $MgSO_2{\cdot}7H_2O$, NaCl and α-chetoglutarate of analytical grade were purchased from Sigma-Aldrich. Pb, Cd and Cr standard stock solutions (1000 ppm) were provided by Merck (Darmstadt, Germany). Nitric acid 70% and ultrapure water for trace metal analysis were provided by J.T. Backer (Mallinckrodt Backer, Milan, Italy).

### 2.2 Microbiological analysis

#### *2.2.1 Culture medium*

A synthetic culture medium (SCM) containing α-chetoglutarate as the only carbon source was used (Pizzimenti et al. 1983) because this source of carbon results in a substantial production of prodigiosin. The medium was constituted by adding 80 ml of each component (Na α-chetoglutarato 0.45%, $K_2HPO_4/KH_2PO_4$ 1%, $NH_4Cl$ 0.2636%, $FeCl_3$ 0.0025%, $MgSO_4{\cdot}7H_2O$ 0.05%, NaCl 0.35%); the pH of the medium was 6.5. To carry out the experiment, sterile culture medium of SCM was added with Pb (0.025 mg/ml), Cd (0.100 mg/ml) and Cr (0.045 mg/ml),

selected after minimum inhibitory concentration (MIC) determination for each metal.

*2.2.2 Bacterial strains and growth conditions in pre-inoculation*

Before the analysis, bacterial strain of *S. marcescens* was grown in a flask containing tryptic soy broth (TSB) culture medium to 28°C for 24 h. This pre-inoculation, needed to obtain bacterial growth in exponential phase with a high metabolic activity, was standardized using a spectrophotometer (Photo Analyzer FT-2, AMS) to 540 nm. The optical density obtained was 0.1030, corresponding to bacterial growth in the pre-inoculation of $15 \times 10^6$ bacteria.

*2.2.3 MIC determination for each metal*

To determine the ·MICs for each metal, 5 μl of *S. marcescens* strain grown in exponential phase and standardized to 28°C was put into SCM, containing increasing concentrations of metals (from 0.20 to 0.00625 mg/ml for Pb; from 0.20 to 0.025 mg/ml for Cd; and from 0.30 to 0.025 mg/ml for Cr). Bacterial growth was examined after 18 h of incubation and the sub-inhibitory concentrations for each metal evaluated through the growth inhibition, the minimum bactericide concentrations (MBC) and the pigment biosynthesis monitoring in medium without metals.

*2.2.4 Pigment biosynthesis monitoring*

To monitor pigment production by *S. marcescens*, bacterial cells were incubated in SCM to 28°C, in the absence of metals and in the presence of sub-inhibitory concentrations for each metal (0.025, 0.100 and 0.045 mg/ml for Pb, Cd and Cr, respectively). Prodigiosin extraction from bacterial cells was carried out according to the method of Pizzimenti et al. (1983). The bacterial cells were separated from the culture medium by centrifugation and extracted with a chloroform–methanol mixture (2:1, *v*/*v*) to isolate the pigmentation. The extract was reduced by a rotavapor and evaporated to dryness; finally, the residue was recovered with isopropyl alcohol. Pigment production was monitored for 120 min, in the absence and the presence of the metals analysed, and measured with a spectrophotometer (PHOTO ANALYZER FT-2, AMS) to $\lambda$ = 466 nm.

2.3 Analysis in atomic absorption spectroscopy

*2.3.1 Sampling*

Strains of *S. marcescens* were incubated in SCM culture medium added with sub-inhibitory concentrations for each metal (0.025, 0.045 and 0.045 mg/ml for Pb, Cd and Cr, respectively) for 120 min. Bacterial biomasses were taken at intervals from 5 ml of medium and then separated by centrifugation at 4,000×*g* for 15 min; finally, all bacterial pellets obtained were washed with distilled water.

Atomic absorption spectroscopy (AAS) analysis was conducted on bacterial pellets of *S. marcescens* ($n$=13) taken each 5 until 60 min and each 10 min until 120 min, the corresponding waters used for their washing ($n$=13) and the relative culture medium ($n$=13). All samples were preserved in PET containers and frozen at −20°C until analysis in AAS to stop metabolic activities.

*2.3.2 Sample preparation and analysis*

Aliquots of 500 μl of culture medium and washing waters for each time were digested in 2 ml of $HNO_3$ (70%, *v*/*v*) for 4 h, whereas bacterial pellets were treated with 1 ml of $HNO_3$ (70%, *v*/*v*) for a night. After digestion, all the samples were mineralized in a microwave oven (model MDS-2100, CEM corporation, Matthews, NC, USA; power, 950 ± 50 W at 100%) and Teflon™ PFA reactors, equipped with a system of pressure regulation (by means of a vessel acting as a sensor). To check possible contamination, analytical blanks were prepared. After the mineralization, all samples and analytical blanks were brought to a volume in a glass volumetric flask (25 ml for culture medium and bacteria samples, 10 ml for wash water samples) with ultrapure water. All the glassware used were treated with a dilute solution of $HNO_3$ (0.1%, *v*/*v*) to prevent contamination.

The concentrations of Cd, Pb and Cr were determined by analysis in graphite furnace (GF-AAS) carried out with an AA Varian model 220/Zeeman spectrophotometer (Varian Australia, Mulgrade, Victoria, Australia), equipped with a single-element hollow cathode lamp and a Varian PSD autosampler, using Zeeman effect background correction, according to Naccari et al. (2009).

The quantification of all elements was performed using the external standard method, and the data obtained for each metal are the mean value of four determinations.

The analytical method was submitted to validation according to the AOAC (1994) guidelines. The linearity was >0.997 for all metals, the precision ranged from 0.255 to 1.462, and the detection limits corresponded to 6.01 pg/ml, 0.078 pg/ml and 1.5 ng/ml for Pb, Cd and Cr, respectively.

Good laboratory practice was applied throughout and procedural blanks were also analysed.

2.4 Data analysis

Data are expressed as the mean±SD of at least four determinations. To study the differences in adsorption and discover the kinetic models of the biosorption process, data of metal uptake on bacterial cells were correlated by either the

Lagergren pseudo-first-order and Ho pseudo-second-order equations.

## 3 Results

### 3.1 Kinetics of growth of *S. marcescens* in the presence of metals

The behaviour of *S. marcescens* in the absence and in the presence of sub-inhibitory concentrations of Pb, Cd and Cr until 120 min is reported in Fig. 1. The growth curve in the absence of metals was used as a control with respect to the growth trend of bacteria in the presence of the sub-inhibitory concentration of each metal, corresponding to 0.025, 0.100 and 0.045 mg/ml for Pb, Cd and Cr, respectively (Table 1).

The preliminary pre-inoculation of *S. marcescens* strain in TSB culture medium was conducted to evaluate that, in this condition, bacteria were full of enzymatic systems and then went on to the exponential phase also during the metabolic activity. In the absence of metals, in fact, the growth curve of *S. marcescens* strain presents an exponential growth phase from 50 to 120 min. In the presence of Pb, the growth of *S. marcescens* is constant until 20 min; from 20 until 120 min, it decreases significantly and the bacterial death is evident at 120 min. Relating to Cd, it is possible to observe the vitality of the strain until 120 min without an exponential growth. In the presence of Cr, the growth decreases significantly from 10 min; the bacterial death appears at 110 min.

### 3.2 Production/inhibition of pigment

The influence of metal adsorption on pigment biosynthesis by *S. marcescens*, monitored in the presence and absence of metals, is reported in Fig. 2. Analysing the figure, it is possible to observe that, in the absence of metals, 'prodigiosin' production is constant from time 0 until 120 min (range, 225–305 μg/ml), whilst in presence of all metals, it decreases after a few minutes, with different trends. In particular, the presence of a sub-inhibitory concentration of Pb (0.025 mg/ml) in the medium causes a significant reduction of pigment production until 80 min (range, 225–5 μg/ml) and then stopped. In the presence of a sub-inhibitory concentration of Cd (0.100 mg/ml), the pigment production decreases in the first minutes but goes out until 120 min (range, 225–31 μg/ml). The sub-

**Table 1** Determination of MIC for Pb, Cd and Cr by *S. marcescens* growth and pigment biosynthesis monitoring

| Metal | Metal conc. (mg/ml) | Control | Bacterial growth/death | Pigment production/inhibition |
|---|---|---|---|---|
| Pb | 0.20 | + | − | − |
| | 0.10 | + | − | − |
| | 0.05 | + | − | − |
| | 0.025 | + | + | + |
| | 0.0125 | + | + | + |
| | 0.00625 | + | + | + |
| Cd | 0.20 | + | − | − |
| | 0.15 | + | − | − |
| | 0.100 | + | + | + |
| | 0.050 | + | + | + |
| | 0.045 | + | + | + |
| | 0.030 | + | + | + |
| Cr | 0.20 | + | − | − |
| | 0.10 | + | − | − |
| | 0.050 | + | − | − |
| | 0.045 | + | + | + |
| | 0.030 | + | + | + |
| | 0.020 | + | + | + |

+: bacterial growth and pigment production;
−: bacterial death and pigment inhibition

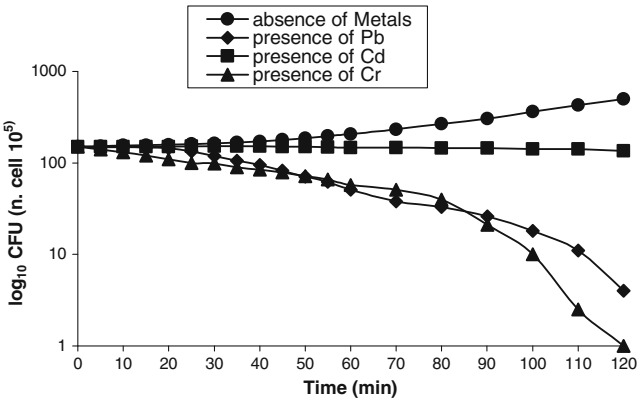


**Fig. 1** Curve of *S. marcescens* growth in the absence and presence of the sub-inhibitory concentration of each metal

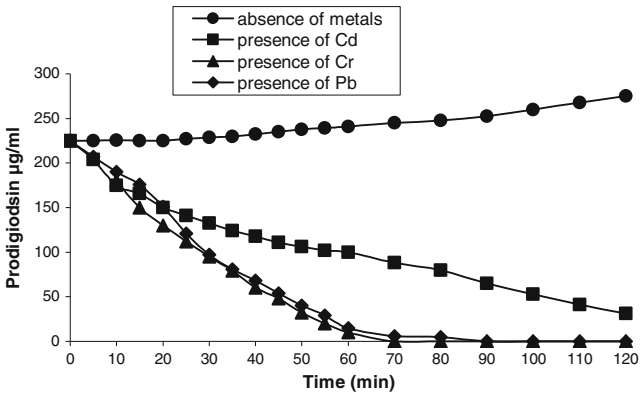


**Fig. 2** Pigment biosynthesis monitoring in *S. marcescens* strain in the absence and presence of the sub-inhibitory concentration of each metal

inhibitory concentration of Cr (0.045 mg/ml) causes a decrease of prodigiosin production, which is inhibited at 70 min (range, 225–15 μg/ml).

### 3.3 AAS determination of metal biosorption

The trends of metal concentration in bacterial pellets, waters used for their washing and the SCM culture medium are reported in Fig. 3.

Relating to *S. marcescens* cells, Pb concentrations were highest of all the metals analysed, which increased slowly from 10 min (0.013 μg/g) until a maximum value of 0.0213 μg/g at 60 min, then decreased to 0.164 μg/g at 120 min. Cd concentrations presented higher values at 10 min (0.182 μg/g) and 20 min (0.185 μg/g), then decreased constantly until 120 min to 0.097 μg/g. Cr levels were intermediate: at the start time of 10 min, the value is 0.114 μg/g, which increased until a maximum of 0.176 μg/g to 60 min and then decreased at 120 min to 0.105 μg/g.

In washing waters Pb levels (range, 0.2–1.52 μg/ml) were lower than the other metals analysed, which increased slowly upon reaching to 1.52 μg/ml at 90 min and decreased to 1.49 μg/ml at 120 min. Cd concentrations were the highest (range, 10.2–16.8 μg/ml); the high value of 16.58 μg/ml is obtained at 20 min, is constant until 30 min, and then decreased to 10.20 μg/ml at 120 min. Cr concentrations were low (range, 1.4–3.8 μg/ml) and the trend was similar to Pb; in fact, the levels increased at 30 min until a maximum value of 3.8 μg/ml at 60 min and decreased to 3.10 μg/ml at 120 min.

In the medium culture at the start time (0 min), the concentration corresponded to the MIC for each metal. For Pb, the trend of metal removal by medium decreased from 10 until 40 min from a value of 23.4 to 3 μg/ml, then increased to 10.3 μg/ml until 60 min; from 70 to 120 min, the value was constant, from 14 to 16 μg/ml. Cd concentrations were the highest (range, 45–100 μg/ml), and the values increased constantly until 120 min, reaching 93 μg/ml. Cr concentrations decreased from 45 μg/ml at 0 to 14 μg/ml at 60 min, then increased slowly to 26.5 μg/ml at 120 min.

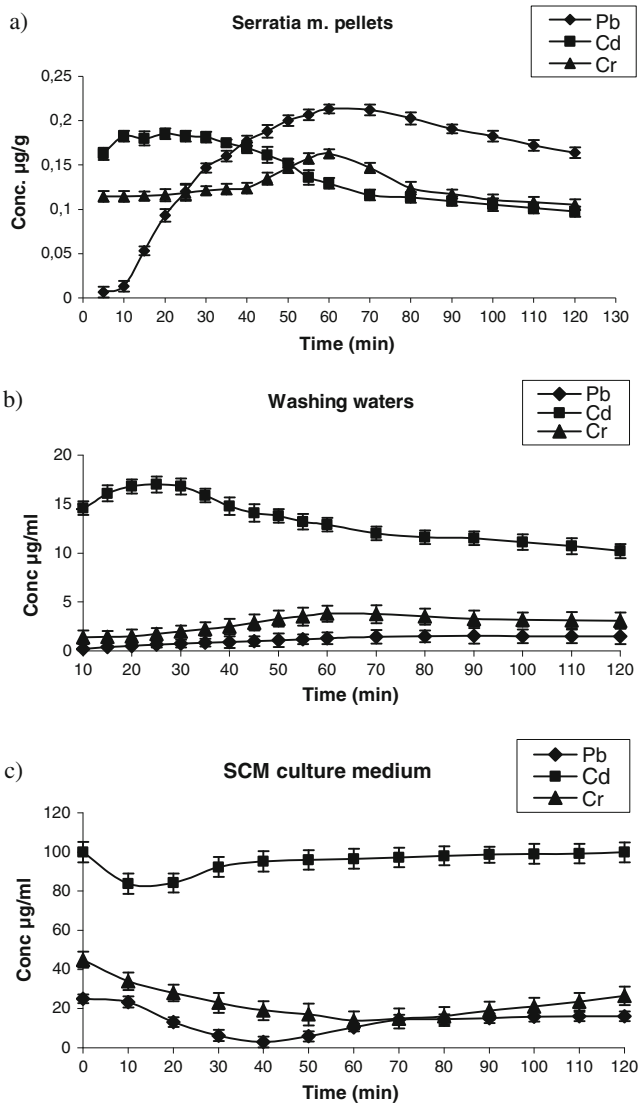


**Fig. 3** Metal concentrations in *S. marcescens* pellets, washing waters and SCM culture medium monitored after incubation in the presence of the sub-inhibitory concentration of Pb (0.025 mg/ml), Cd (0.100 mg/ml) and Cr (0.045 mg/ml)

### 3.4 Kinetics of metal uptake

The kinetic mechanism of metal adsorption was studied to evaluate the uptake rate of *S. marcescens*, the effectiveness of metal removal and the possible release of metals.

In order to describe the kinetic models of the biosorption, data of Pb, Cd and Cr adsorptions by *S. marcescens* have been modelled by Lagergren pseudo-first-order kinetics (1) and Ho pseudo-second-order kinetics (2). These equations are the most widely used rate models in liquid phase metal sorption processes (Bishnoi et al. 2007; Benguella and Benaissa 2002):

$$\log(q_e - q_t) = \log q_e - K/2.3 \times T \quad (1)$$

$$T/q_t = 1/2K' \times q_e^2 + T/q_e \quad (2)$$

where $K_L$ (min) is the Lagergren rate constant of adsorption; $K'$ (g μg$^{-1}$ min$^{-1}$) is the Ho pseudo-second-order rate constant of adsorption; and $q_e$ and $q_t$ are the amounts of metal adsorbed (μg/g) at equilibrium and at any time $t$; respectively.

The $K_L$ value, calculated from the graph plotted between $\log(q_e - q_t)$ and time $T$ (min) and the correlation coefficients ($R^2$), indicated the adequate model to describe the kinetics (Fig. 4). In particular, these equations provide the

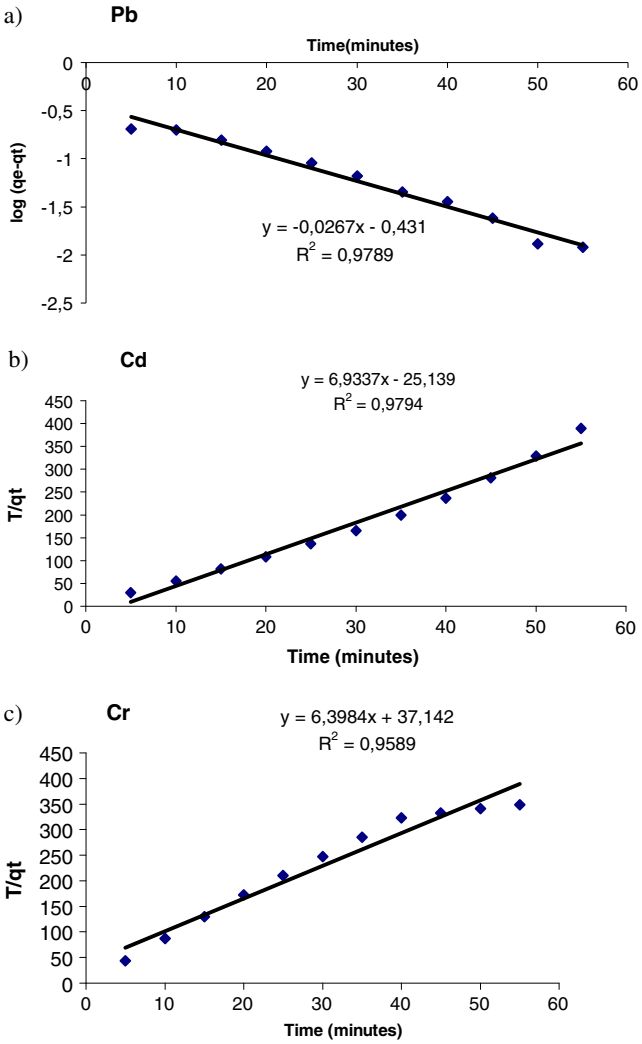


**Fig. 4** Kinetics of metals removal by *S. marcescens* by Lagergren pseudo-first-order for Pb (**a**) and Ho pseudo-second-order for Cd (**b**) and Cr (**c**)

best overall prediction for the biosorption: For Pb, the relationship between concentration and rate follows a kinetic of pseudo-first-order, whilst the pseudo-second-order reaction rate model adequately describes Cd and Cr biosorption kinetics.

## 4 Discussion

The results obtained show a chemical–physical interaction between the metals analysed and *S. marcescens*, underlining a different bacterial behaviour in the presence of Pb, Cd and Cr.

From the microbiological analysis, the kinetics of growth in the presence of metals show that *S. marcescens* can tolerate sub-inhibitory concentrations of these contaminants; for Pb and Cr, the stop time of analysis corresponds to bacterial death, whilst for Cd strain vitality is constant at 120 min. The biosynthesis of the red pigment 'prodigiosin' was monitored to evaluate the cytotoxicity of metals on *S. marcescens*, and effectively, the trend of production demonstrates a different tolerance of bacteria to metals. In fact, prodigiosin biosynthesis decreased after a few minutes from the start time of analysis, but in the presence of Pb and Cr is stopped completely at 80 and 60 min, respectively, whilst in presence of Cd is reduced but went on for all times of the analysis. These data underline probably a greater toxicity of Pb and Cr than Cd, more tolerated by *S. marcescens*, as confirmed by the kinetics of growth that goes on only in the presence of Cd for all times of the analysis.

The trends of metal concentration in bacterial pellets, washing waters and culture medium obtained from the AAS analysis confirm the biosorption by *S. marcescens* but show a different bacterial behaviour in the presence of the three metals. Analysing the bacterial pellets, it is possible to observe that for Pb, the assumption is slow but more than the other metals, reaching the highest levels that it held until the stop time of analysis. For Cd, the assumption starts after a few minutes of incubation and presents the highest concentration until the stop time of the analysis. For Cr, metal–bacteria interaction is gradual; the maximum levels are reached at 60 min and then decrease upon bacterial growth and pigment production. These metal trends of adsorption are similar to those found in waters used for bacterial washing, but inverse with respect to those present in the SCM culture medium.

The study of kinetics of adsorption (from 10 to 60 min), in addition, confirms the ability of *S. marcescens* to remove Pb, Cd and Cr from the medium according to a different rate order model, whilst from the analysis of metal desorption (from 60 to 120 min), there appears a higher effectiveness of *S. marcescens* in retaining Pb. This different amount of metal adsorption/desorption by *S. marcescens* should be attributed to bacterial defensive mechanisms, such as metal-thioneines, active in regulating metal transport in and out of bacterial cells, considering the vitality/deadly of cells in the presence of the metals analysed and the different metal toxicities. Moreover, these results demonstrate that *S. marcescens* tolerates the presence of metals and interacts with them, also if Pb and Cr result to be more toxic for cells than Cd, as confirmed by the *S. marcescens* growth curve and the pigment production trend.

Relating to the possible mechanism of biosorption, the manner by which microorganisms effect changes in metal speciation and mobility are fundamental components of the biogeochemical cycles for metals (Gadd 2000). Data in literature about metal–microbe interactions, metal sorption and metal desorption processes from bacteria (Srivastava et al., 2008) show that positively charged metal ions are sequestered primarily through the adsorption of metals to

the negative ionic groups on cell surfaces, the polysaccharide coating present in most forms of bacteria or other extracellular structures, such as capsules or slime layers. The binding sites for metals on microbial cell surfaces usually are carboxyl residues, phosphate residues, SH groups or hydroxyl groups; non-essential metals bind with greater affinity to the SH group (Srivastava et al., 2008). Chelating agents, such as siderophores and other ligands are produced by microorganisms to mobilize and scavenge metals, with the formation of the siderophores complex (Edberg et al. 2010). Also, metallothioneins (MT), essential and non-essential metal-binding proteins, play an important role in the detoxification of toxic elements. MT gene transcription, in fact, is induced by heavy metals through metal response elements, and the overexpression of MT genes after exposure to metal ions is responsible for the resistance to heavy metal toxicity in bacteria (Rifaat et al. 2009). The same researchers explain metal assumption by *S. marcescens* as a secondary activity of the reductase enzyme with the physiological role for Cr (Campos et al. 2005), involving RND-driven trans-envelope efflux systems to detoxify the periplasm by exporting toxic cations such as Co and Ni (Marrero et al. 2007). Considering the results obtained in this study and the chemical behaviour of the metals analysed, it is possible to hypothesize that the mechanism of biosorption by *S. marcescens* could be due to the absorption by metal-thioneines or siderophores produced by bacterial cells and be carried out through processes of intracellular chelation.

## 5 Conclusions

This study confirms that bacteria are highly effective in sequestering metals and that the interactions between minerals and microbes play an important role in regulating environmental metal mobility. From the results obtained in this study, *S. marcescens* shows its ability in removing metals from a polluted environment and a low resistance to metal cytotoxicity on prodigiosin biosynthesis and appears to be a possible bioaccumulator and bioindicator of Pb, Cd and Cr environmental pollution. Consequently, it is possible to suggest the use of this bacterium to realize quite effective and low-cost metal bioremediation processes, especially to carry out Pb removal because of its higher effectiveness in desoprtion of this metal.

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