# HEAVY METALS IN THE ENVIRONMENT

*Microorganisms and Bioremediation*

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CRC Press
Taylor & Francis Group
Boca Raton London New York

CRC Press is an imprint of the
Taylor & Francis Group, an **informa** business
A SCIENCE PUBLISHERS BOOK

CRC Press
Taylor & Francis Group
6000 Broken Sound Parkway NW, Suite 300
Boca Raton, FL 33487-2742



Printed on acid-free paper
Version Date: 20171114

International Standard Book Number-13: 978-1-1380-3580-5(Hardback)



**Library of Congress Cataloging-in-Publication Data**

Names: Donati, Edgardo R., editor.
Title: Heavy metals in the environment : microorganisms and bioremediation / editor, Edgardo R. Donati, National University of La Plata, La Plata, Argentina.
Description: Boca Raton, FL : CRC Press, 2018. | "A science publishers book." | Includes bibliographical references and index.
Identifiers: LCCN 2017044396 | ISBN 9781138035805 (hardback)
Subjects: LCSH: Heavy metals--Environmental aspects. | Bioremediation.
Classification: LCC TD196.M4 H4295 2018 | DDC 628.4/2--dc23
LC record available at https://lccn.loc.gov/2017044396

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# PREFACE

Heavy metals and metalloids are released into the environment from anthropogenic activities without rare or null concern or government regulation in many countries. Sources of such pollutants are industrial effluents, municipal waste treatment plants, landfill leaching and mining activities, among others. Since heavy metals cannot be degraded, they persist and are accumulated over time, increasing the human exposure and causing serious negative environmental consequences. Although numerous physicochemical technologies have been developed in the last decades to remediate sites contaminated with heavy metal(loid)s, most are expensive and/or inefficient at low metal concentrations and large polluted areas. Hence, many new treatments have emerged in the last few decades.

In bioremediation processes, biological technologies are used to remediate contaminated environments. These processes offer high specificity in the removal of some particular heavy metal(loid)s of interest while also offering operational flexibility. In the 17 chapters written by experts in the field, this book deals with different approaches to the topic.

The first part comprises some aspects of the interaction between microbial communities and microorganisms and heavy metal(loid)s, including mechanisms of resistance to such pollutants. In the second part, different strategies for bioremediation are described: biosorption and bioaccumulation, bioprecipitation, biosolubilization, and also phytoremediation. The third part elucidates particular bioremediation cases for some of the most relevant heavy metal(loid)s: arsenic, cadmium, cobalt, copper, lead, and mercury. The last part comprises three chapters with field applications including an application using wetlands. These 17 chapters configure a comprehensive understanding of this area including some novel and interesting approaches to the topic.

I would like to acknowledge the efforts of all the contributors for bringing the book to fruition. The continued assistance of the Editorial Department of CRC Press is also highly appreciated.

**Edgardo R. Donati**

# CONTENTS

# CHAPTER 12

# Process Oriented Characterization in Bioleaching Co-Cu Minerals

*Guy Nkulu*[1] and *Stoyan Gaydardzhiev*[2,*]

## 1. Introduction

The copper-cobalt mineralization of the Katanga basin belonging to the Central African Copper belt and situated between the Democratic Republic of Congo (DRC) and Zambia is famous with its mineral reserves. Apart from the enormous copper deposits, more than 40% of the world cobalt production originates from this region (Laurence, 2005; Yager, 2014). Historically, the polymetallic sulphide ores and concentrates from this region have been treated through traditional pyrometallurgical and acid leaching routes. Nevertheless, environmental constraints imposed by the recent mining legislature coupled with the rising costs of the established metal extraction methods have led to the abandoning of several potentially exploitable deposits. This is the case of Kamoya deposit, characterized by stratified type ore bodies with uniformly disseminated carrollite mineralization. During the times of operation, the ore has been processed through sulphating roasting followed by hydrometallurgy for recovery of copper, cobalt, and nickel. During 1998, however, the operations in the mine had been seized due to both technological issues (e.g., Ni elimination

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from the PLS) and financial downturns. Later on, exploratory studies were launched to find alternative and economically viable ways to extract remaining metals (cobalt mainly) from the ore and from the surrounding tailings (Kitobo, 2009). These studies have been realized in majority on lab scale with very a few of them being further up-scaled. Given the fact that carrollite is widely present elsewhere in the polymetallic deposits of the Katanga metallogenic zone, there is a strong incentive to render biometallurgy, a commercially justifiable option for cobalt extraction. In order to meet this need, the dissolution mechanisms of the carrollite have to be identified better. While bioleaching reactions for copper minerals are broadly-well known, there is no doubt that carrollite is likely to undergo bio-oxidation at a rate and at an extent which are not known.

When polymetallic sulphides are subjected to bioleaching, the microorganisms could be found either in the liquid phase (refereed as planktonic MO) or attached to the surface (what we called fixed or anchored to the surface MO), the latter ones forming the biofilm. Their importance in view revealing the responsible leaching mechanisms have stimulated numerous studies for counting, characterizing and identification, e.g., Q-PCR, MPN (Beach and Sunner, 2004; Kinzler et al., 2003; Dziurla, 1995, 1998). The ultimate aim has been to design the optimum proportion of microorganism members that a used consortium for bioleaching should contain.

In the studied case having an exploratory character, the initial strategy was to estimate the number of "planktonic"microorganisms met in solution and of those "fixed" or anchored on the carrollite surface in the course of the leaching duration. For the latter, we have chosen a methodology based on physicochemical desorption, which is known by its simplicity and accuracy and at the same time is able to distinguish the two types of anchored bacteria: transiently-bound (reversibly detachable) and strongly-bound. The method was originally developed by Monroy et al. (1993) and used by Dziurla (1995, 1998). In our case, the estimation of the transiently bound (reversibly detachable) microorganisms has been realized by gentle wash-out using 9K solution, while the strongly-adhered ones—by means of a "Tween 80" detergent which is a combination between anionic and non-ionic surfactants. Figure 1 illustrates the experimental protocol used for the estimation of the number of fixed microorganisms.

The findings coming from bacteria counting are complemented by observation on the mineral surface in order to trace the changes resulting from bacterial presence and to link them to the basic bioleach amenability of the mineral.

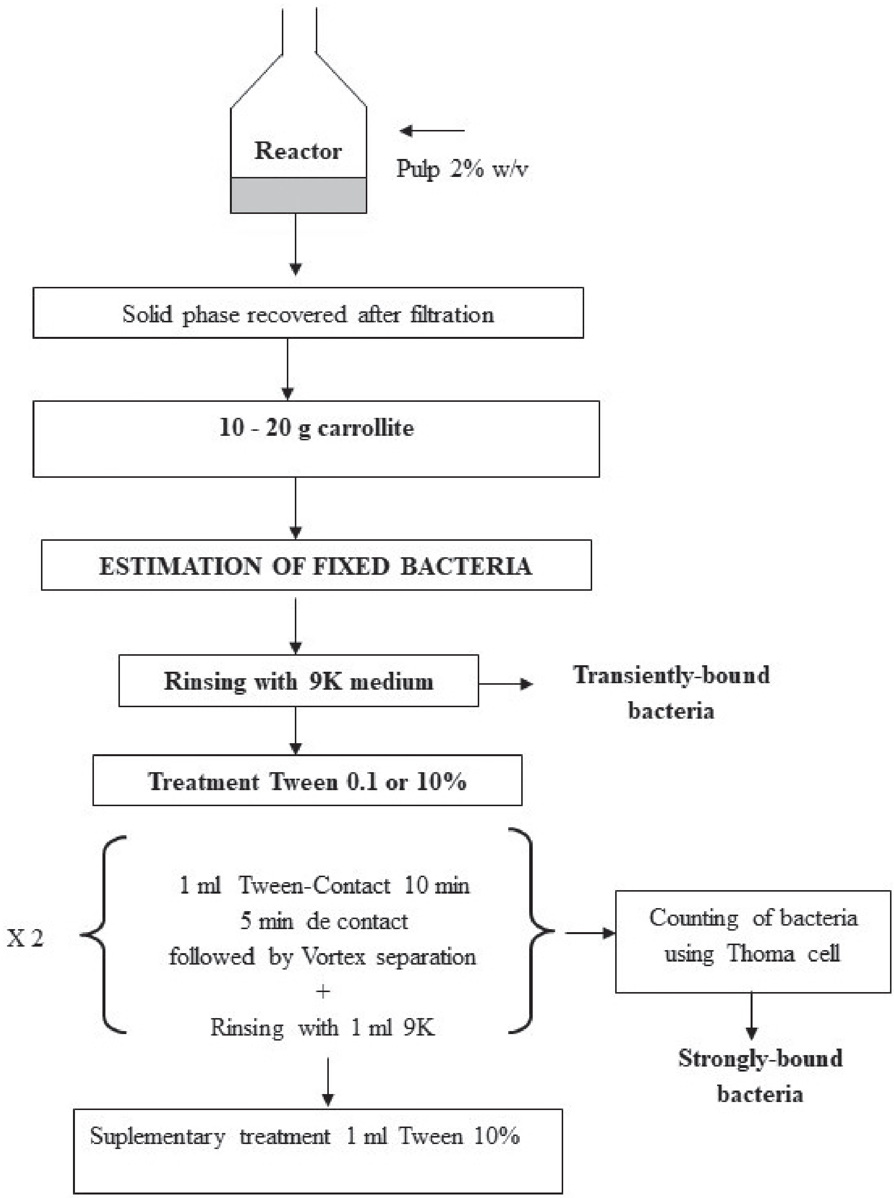


**Figure 1.** Experimental set-up for estimation of surface-fixed microorganisms.

## 2. Materials and methods

High purity carrollite samples accompanied by their dolomitic gangue were handpicked from the rich mineralized zones of the Kamoya deposit. The carrollite mono-crystals have been further fragmented and prepared as to render them suitable for bio-leaching. The consortium comprised three different mesophilic iron and sulphur oxidizing bacteria, e.g., *A. ferrooxidans*, *L. Ferrooxidans,* and *A. thiooxidans* isolated in Bulgaria. The mixed culture was adapted to grow on the solid substrate before being used further. The entire bioleaching procedure, together with counting and characterization of methodologies, is described in our previous publications (Nkulu et al., 2013, 2015).

## 3. Role of microorganisms: experimental results

### *3.1 Evolution of "fixed" and "planktonic" microorganisms*

The grow rate of the "fixed" microorganisms as function of the leaching duration has been followed through implementing the physicochemical desorption protocol described above, while the number of "planktonic" microorganisms was counted directly in the leach solutions. Three distinguished phases characterized by varying number of bacteria in solution and on the surface were detected as follows:

Phase 1—that lasted until approximately day 5, during which the concentration of "planktonic" bacteria remained nearly constant (± $10^7$ cells/mL). It could be argued that this period coincided with the "latent" phase of bacterial activity. During this phase the number of fixed bacteria (both strongly and reversible detachable) increased slightly.

Phase 2—after day 5, we observed strong increase in the "planktonic" bacteria population. We could infer that bacteria started to grow exponentially due to ferric iron reduction taking place continuously at the mineral surface and leading to concomitant increase in ferrous iron concentration in the solution. As a result, the numbers of "planktonic" microorganisms grew exponentially. In parallel, the generated extracellular polymeric substances (EPS) started to form biofilms. At day 15, the number of planktonic microorganisms reached $4.1 \times 10^{10}$ cell/mL, while the number of the surface-fixed ones approached $5.5 \times 10^8$ cells/mL.

After day 20, the third and last phase could be distinguished. This period was characterized by a sharp drop in the number of fixed microorganisms on the expense of just negligible decrease of the planktonic ones. Two reasons could explain these phenomena; (1) either there is lack of nutrient medium being consumed through the jarosite formation or (2) the concentration of ferrous ions is reduced by precipitation or jarosite formation. The latter effect seems plausible since the mineralogical analysis of the leached residue had confirmed jarosite presence—Fig. 2, jarosite marked as J.

In parallel to the estimation of the number and type of microorganisms as functions of the leaching duration, the observations on selected mineral grain surfaces have been useful in defining the role which the fixed microorganisms play during the bioleaching. In the figures which follow, few exemplified situations of microorganisms anchored on the surface of the carrollite grains are illustrated. The perusal of the mineral grain SEM image shown in the left side of Fig. 3, witnesses anchored microorganisms together

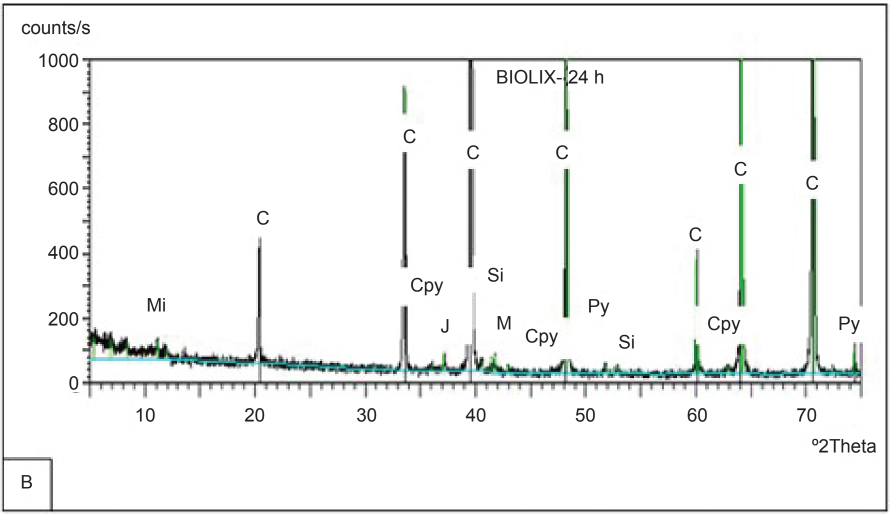


**Figure 2.** Typical mineralogical composition of carrollite residue after 25 days of bioleaching.

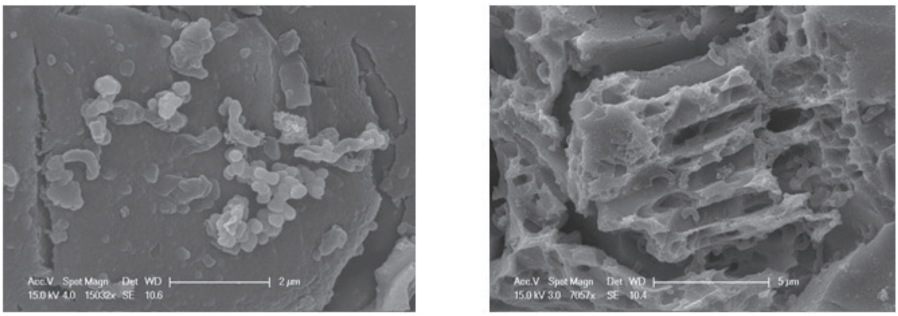

**Figure 3.** SEM images of mineral grains occupied by bacterial cultures. Left—mineral grain after 12 days leaching; Right—mineral grain after 20 days leaching.

with iron oxides globules and EPS. Aggregates possibly consisting of mineral particles and organic substances are seen. In the right image, mineral grains heavily degraded by the microorganisms met in close proximity do appear. These microorganisms contribute to the generation of visible precipitates as well which most likely do not have chemical origin but rather are formed under bacterial action. The fragments which are composed from mineral particles and organic compounds could be viewed as intermediate sources of energy, thus, supporting the important role of the EPS during the carrollite bioleaching. These observations are in agreement with the model proposed by Schippers et al. (1996) describing the situation where the EPS containing ferric ions have played an important role during pyrite oxidation by *A. ferrooxidans*.

It is known that *L. ferrooxidans* cells tend to accumulate high concentration of $Fe^{3+}$ within the EPS they produce (Rojas-Chapana and Tributsch, 2004). Therefore, it could be argued that cells abundantly seen at the left image in Fig. 4 do belong to this genus. However, due to differences in the adhesion energy between both bacterial types, *A. ferrooxidans* cells are likely to be associated with corrosion pits close to edges (Edwards and Rutenberg, 2001). This situation could be spotted at the right image shown in Fig. 4.

The performed microscopic observations allow us to likewise draw some clues about the life-cycle of the microorganisms. It could be postulated that the evolution of bacterial population follows two distinguished pathways. The first phase, lasting up to day 10, could be considered as maturation stage when bacteria are attaching to the surface and biofilms start emerging. During this period, the anchored bacteria on the surface eventually leave their remnants on it—Fig. 5 on the left. Moreover, visible traces of bacterial attacks toward preferential zones and corrosion pittings could be seen. The second phase, between day 10 and 20, essentially corresponds to a biochemical attack when the mineral surface begins to degrade heavily—Fig. 5 on the right. Physical detachment of mineral's micro-particles takes place followed

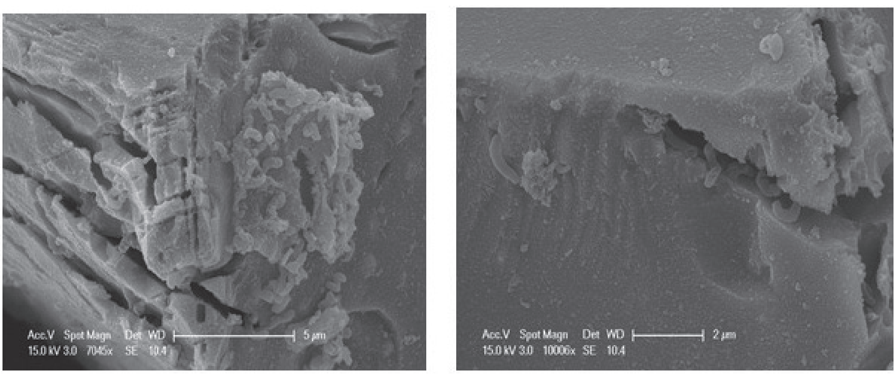


**Figure 4.** SEM images of carrolite surface after 15 days of bioleaching.

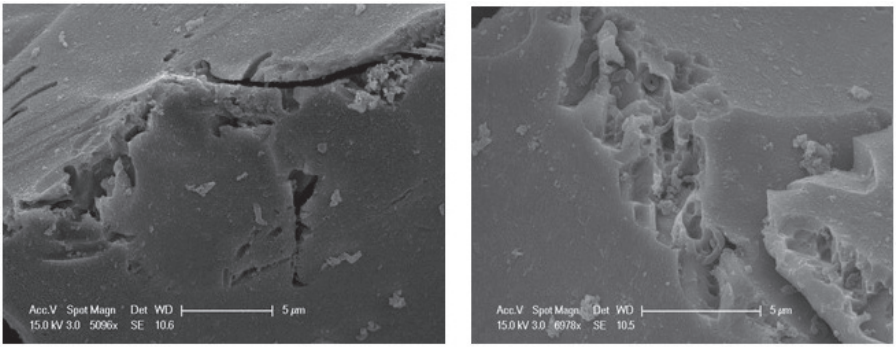


**Figure 5.** SEM images of mineral grains occupied by bacterial cultures. Left—mineral grain after 6 days bioleaching; Right—mineral grain after 15 days bioleaching.

by the emerging of aggregates containing mineral-organic substances. These phenomena are naturally enhanced by bacterial presence.

### *3.2 Role of the contact bacteria/mineral*

Several studies aiming to define the role of the contact between microorganisms and minerals have pointed out the contribution of the said contact towards efficient bringing of metals in solution. Nevertheless, published results suggest that the leaching efficiency also depends to larger extent on the nature of the metallic sulphides being tested (Konishi et al., 1992; Pistaccio et al., 1994; Porro et al., 1997).

In the mentioned studies, the role of the contact has been often investigated through purposely designed set-ups enabling physical separation between bacteria and mineral. For example, results reported by (Pogliani et al., 1990) for the CuS-*T. ferrooxidans* system were obtained by implementing dialysis sacs. The importance of the direct contact for the studied case has been demonstrated but at the same time the role of the ferrous iron re-oxidation is equally emphasised.

Other studies (Larsson et al., 1993) have shown that in the case of pyrite oxidation by thermophilic archeon *Acidianus brierleyi*, an efficient leaching was only possible if a direct contact between cells and mineral substrate was established.

For the case of carrollite bioleaching in order to experimentally follow the role of bacteria-mineral contact, a double-compartment reactor fitted with microporous membrane has been designed. The objective was to physically separate the microorganisms from the mineral particles and at the same time to allow free exchange of ions and soluble products between both sides of the reactor. This aim was realized by placing a membrane having openings smaller than the size of bacteria. In such a way, the direct action of the fixed bacteria could be identified and compared to the situation where only ferric ions are allowed to enter into contact with the mineral. An intermediate situation simulating semi-contact has been realized as well where half of the mineral mass has been placed in contact while the other one was separated from the microorganisms. Figure 6 shows the methodological set-up and the protocol which was followed to this end.

Figure 7 reports the extraction degree of Co and Cu with time for the cases of: full contact (b); lack of contact (c) and semi-contact (d) between bacteria-mineral as illustrated in Fig. 6. The immediate impression from the cobalt and copper extraction curve trends suggest quite a similar trend for the three cases being studied. In terms of leaching kinetics, one could note three separate zones being function of the leaching duration: the first one between days 0–13, the second one between days 12–20 and the third one between

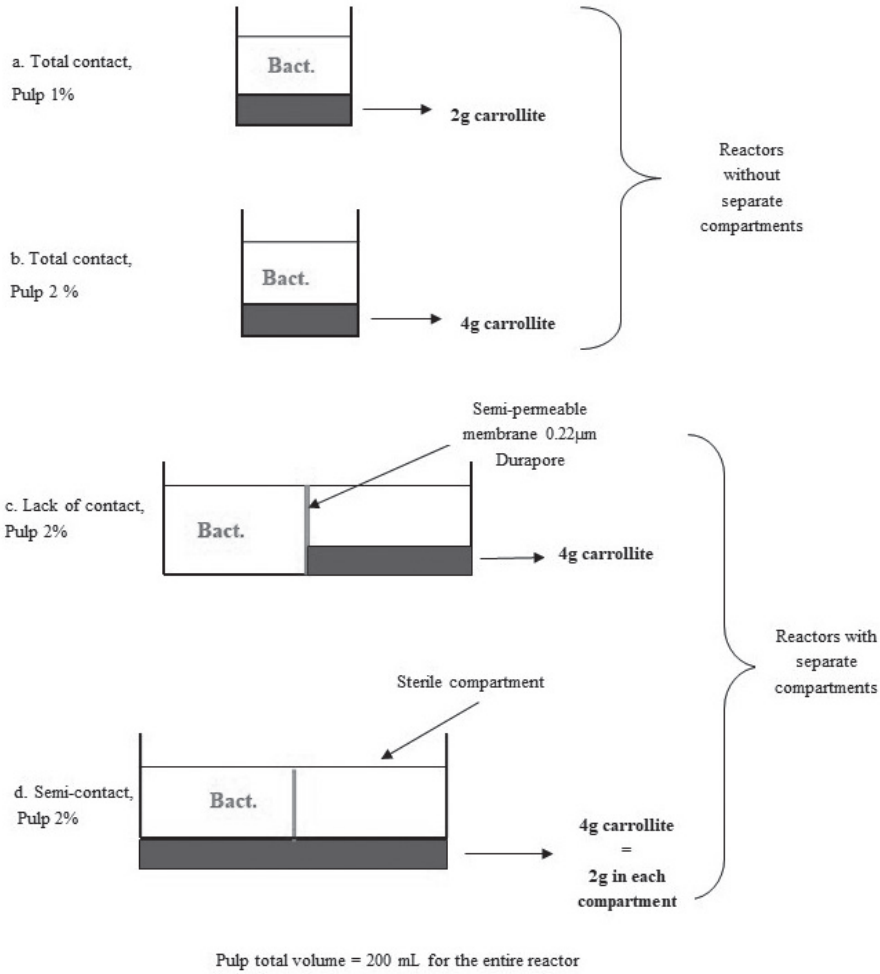


**Figure 6.** Experimental set-up for simulation of various situation of contact bacteria-carrollite

days 20–30. The first period encompasses both latent and growing phases which are quite evident in the bacteria-mineral total contact mode. Logically, metals recovery in the PLS is much higher in the case of total contact. These findings are in agreement with the published results by Dziurla et al. (1995), where an increased iron solubilization was observed in the case of direct contact between pyrite and *Acidithiobacillus ferrooxidans*, thus confirming the catalytic role of the surface-anchored bacteria. The latent phase in the case of total contact lasts up to day 3 with worth mentioning that during this period the number of fixed bacteria is quite low. Therefore, during this period, the observed leaching of copper and cobalt could be entirely due to the ferric iron accompanying the bacterial inoculum. After day 3 and further to day 13, the rapid raise in metal extraction degree could be attributed to the abundance of ferric iron regenerated by the "planktonic" bacteria. Between days 13–20, a second type of behavior could be identified, where in the case of total contact slight increase in metals recovery was recorded. During this period, the iron oxidation rate (IOR) seems to slow down although the number of "planktonic" bacteria remains constant. However, for the case of non-contact, metal extraction continues to rise being function of the IOR, the latter one being catalyzed by bacteria. It should be noted that the number of bacteria remain nearly constant inside the separate compartment of the reactor where carrollite was absent. This finding suggests that the chemical oxidation of the carrollite continues to progress as long as ferric ions are available in the compartment where only mineral is present, bacteria being absent.

During the third period, covering days 20–30, one could note that in the case of total contact, the metal extraction rate is virtually zero with the sufficient number of "planktonic" bacteria being available. One factor governing this situation could be the excessive formation of jarosite precipitates on the surface of the mineral. These precipitates are limiting the diffusion of the ferric ions towards the surface. For the case of non-contact, the fact that metal leaching continues is supporting the assumption that carrollite solubilization essentially follows chemical route. When comparing

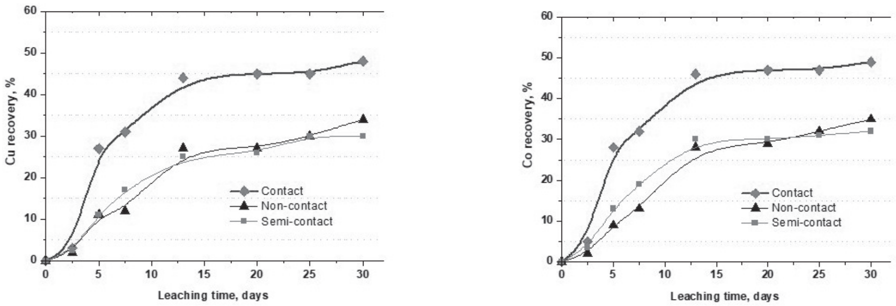


**Figure 7.** Recovery of Cu and Co as function of leaching duration for the case of total contact, semi-contact and lack of contact.

the situations of total- and semi-contact, one could find the important role which the contact bacteria/mineral plays in relation to the leaching kinetics, especially during the initial period (0–13 days).

These findings corroborate well with the results reported by Konishi et al. (1992), indicating almost equal role of the direct and indirect mechanisms during bioleaching of sphalerite concentrate. However, for the case of molybdenite which is refractory to leaching, Pistaccio et al. (1994) have shown that economically acceptable oxidation is achievable only if sufficient adhesion of *A. ferrooxidans* on the mineral surface takes place. In the same context, results communicated by (Porro et al., 1997) have likewise proved the importance of mineral-bacteria contact during the bioleaching of covellite by *T. ferrooxidans*.

If we compare the carrollite behavior in the tested leaching system with the hypothesis of contact mechanism as proposed by Rohwerder et al. (2003), we could infer that in the case of carrollite bioleaching, the main role of the surface-anchored bacteria will be to catalyze ferrous ions oxidation. Moreover, this catalytic role is supposed to be enhanced by the bacteria-generated EPS, creating an adequate microenvironment for the microorganisms and enabling them to contribute towards mineral oxidation. In such a way, being englobed inside its microenvironment, bacteria appear in “indirect” contact with the mineral surface.

## 4. Conclusion

This study has allowed following the evolution of bacterial population and its repartitioning between the solid and the liquid phases. The role which bacteria play in carrollite bioleaching system has been thus clarified. SEM examinations have shown pitting patterns for which specific bacterial species present in the consortium are responsible.

Strong adhesion of bacteria to the surface of the carrolite grains was observed during early bioleach stages, manifesting their non-negligible role in the process. The direct contact has favored carrollite oxidation through electrochemical pathway, at the same time being accompanied by release of ferrous ions, elemental sulphur, or sulphur compounds which accumulate on the surface of carrollite. The generated ferrous ions are further used as an energy source by the planktonic bacteria.

## Acknowledgment

The authors wish to acknowledge the kind contribution of Prof. Chr. Mustin, LIMOS, Univ Lorraine, France, in studying the role of bacteria-mineral contact. Also special thanks to Prof. St. Groudev from the UMG in Sofia, Bulgaria for providing the initial bacterial consortium.

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