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# Biodegradation of free cyanide by bacterial species isolated from cyanide-contaminated artisanal gold mining catchment area in Burkina Faso

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## HIGHLIGHTS

- Cyanide is extensively used for gold extraction in artisanal mining sites.
- Water and soil samples were collected to evaluate site contamination with cyanide.
- Free cyanide was detected in all collected water and soil samples.
- Biodegradation tests were conducted with microorganisms from the contaminated site.
- The isolated species have high potential for cyanide bioremediation application.

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## ABSTRACT

Soil and water samples were collected from a watershed in Burkina Faso where illegal artisanal gold extraction using cyanidation occurs. The samples were used to evaluate cyanide contamination and the presence of cyanide degrading bacteria (CDB). Free cyanide (F-CN) was detected in all samples, with concentrations varying from 0.023 to 0.9 mg $kg^{-1}$, and 0.7–23 μg $L^{-1}$ in the soil and water samples, respectively. Potential CDB also were present in the samples. To test the effective F-CN degradation capacity of the isolated CDB species, the species were cultivated in growth media containing 40, 60 or 80 mg F-CN $L^{-1}$, with or without nutrients, at pH 9.5 and at room temperature. More than 95% of F-CN was degraded within 25 h, and F-CN degradation was associated with bacterial growth and ammonium production. However, initial concentrations of F-CN higher than 100 mg $L^{-1}$ inhibited bacterial growth and cyanide degradation. Abiotic tests showed that less than 3% of F-CN was removed by volatilization. Thus, the degradation of F-CN occurred predominately by biological mechanisms, and such mechanisms are recommended for remediation of contaminated soil and water.

The bacteria consortium used in the experiment described above exist in a Sahelian climate, which is characterized by a long hot and dry season. Because the bacteria are already adapted to the local climate conditions and show the potential for cyanide biodegradation, further applicability to other contaminated areas in West Africa, where illegal gold cyanidation is widespread, should be explored.

## 1. Introduction

Cyanide is a strong toxic chemical frequently used in various industrial processes, including synthetic fibre production, extraction of gold and silver, coal processing and extractive metallurgy (Boucabeille et al., 1994; Mudder and Botz, 2004). In the mining

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industry, cyanide is also widely used to recover gold residues after mercury amalgamation (Carling et al., 2013). The use of cyanide in artisanal small scale gold mining (ASGM) is widespread in West-Africa, particularly in Burkina Faso (Butaré and Keita, 2009). The majority of current mine workers were initially farmers that started to be involved in the activity to complete their revenues during dry season and later as resilience to climate change (Luning, 2006). They have no official authorization to work on the site and are using rudimentary tools and techniques, without any protection. Due to the illegal aspect of the activity, it is extremely difficult to access ASGM cyanidation sites and limited data is available on cyanide use and environmental distribution in ASGM in the literature.

In the natural environment, cyanide can be present in three forms: free cyanide (F-CN), weak acid dissociable cyanide (WAD-CN) and strong acid dissociable cyanide (SAD-CN) (Botz et al., 1995); hydrogen cyanide (HCN), a F-CN component is the predominant form in most natural waters (Doudoroff, 1976; Moran, 1998; Souren, 2000). HCN and other cyano-compounds that liberate F-CN ions are highly toxic to almost all living organisms, and their presence in natural waters and soils can be harmful to aquatic ecosystems and to human health (Clarke and Morna, 2009; Hijosa-Valsero et al., 2013). Most cyanide contamination is linked to gold mining activities (Clarke and Morna, 2009).

Numerous chemical and physical processes have been developed to remove cyanide from contaminated water and soil (Mudder et al., 2001). However their applications were often limited, due to variations in environmental conditions, operational costs and the production of hazardous by-products (Dzombak et al., 1996; Akcil, 2003; Kumar et al., 2013; Botz et al., 2015). On the other hand, biological processes are known to be environment-friendly and cost-effective (Botz et al., 2015) and well adapted for treating the gold mining effluents (Akcil and Mudder, 2003). Kumar et al. (2013) have suggested that the use of microorganisms could be the best alternative to transform cyanide compounds into less toxic compounds in the environment. Many studies have demonstrated the capacity of microorganisms to efficiently degrade F-CN (Boucabeille et al., 1994). CDB use cyanide as a source of carbon and/or nitrogen for their metabolism through enzymatic reactions by destroying the C—N link (Bouari et al., 2013). The reactions produce ammonia, formic acid or formamide, depending on the enzymes present in the microorganisms (Huertas et al., 2010; Luque-Almagro et al., 2011; Kumar et al., 2013). Some CDB will then convert ammonia to nitrite or nitrate (Akcil, 2003). Temperature, pH and nutrient availability are the main parameters that control the efficiency of cyanide degradation (Kumar et al., 2013). The optimum temperature for cyanide degradation was usually around 30 °C (Akcil et al., 2003; Chen et al., 2008; Huertas et al., 2010; Shete and Kapdnis, 2012; Wu et al., 2014). The rate of cyanide degradation is inversely related to temperature, when temperature is varied between 27 °C and 50 °C (Basile et al., 2008). Burkina Faso and neighbouring sub- Sahara countries have a Sahelian climate which is characterized by extremely hot and dry seasons during which temperatures can range from 30 °C to 52 °C. Under these conditions, only specific species of bacteria can survive. Most experiments have been performed using pure bacterial species from GenBank that are not directly related to soil or water in contact with mining activities (Ezzi and Lynch, 2005; Dash et al., 2008; Gurbuz et al., 2009). Boucabeille et al. (1994) and Mekuto et al. (2013) are among the few researchers that have investigated the cyanide degradation capacity of in-situ bacteria collected from electroplating wastewater and from the sludge of mining wastewater storage basin, respectively. One bacteria specie is more used. There are advantages of using a consortium of bacterial species rather than one species. Enzymes in a consortium of bacteria can be stable over long periods (Jandhyala et al., 2005), which ensures high quality and sustainability of the remediation (Basile et al., 2008). On the other hand, the consortium of bacteria could be able to use cyanide as a sole carbon and nitrogen source than the one specie (White and Schnabel, 1998). It will contribute in achieving a high level of cyanide degradation efficiency rate (Mekuto et al., 2013). Several authors have strongly recommended that future research should focus on testing indigenous species (Mudder et al., 2001; Dash et al., 2009) and on using a consortium of bacteria (Mekuto et al., 2013).

During a baseline survey conducted in 2014 at the “Zougnazagmiline” ASGM site, located in the northern part of Burkina Faso, authors have found that the quantity of cyanide used in only one ASGM site could reach easily 20 Kg per week. Furthermore, cyanide-containing leachate is released into the environment without treatment.

The purpose of this study is then to look for the contamination of an uncontrolled artisanal mining site by F-CN at catchment level and to test the potential for cyanide biodegradation of locally available bacterial species that are already adapted to these difficult environmental conditions.

WAD-CN is the most commonly used form of cyanide at ASGM site. Under moderate acidic pH conditions, WAD-CN can be easily converted to F-CN (Ghosh et al., 2006), which is the most toxic form of cyanide in the environment (Botz et al., 2015). Therefore, this study focused on the removal of F-CN from the contaminated site.

## 2. Material and methods

### 2.1. Site description and sampling location and procedures

The “Zougnazagmiline” site is located in the Center-North Region of Burkina Faso, in the District of Bouroum, about 300 km far from Ouagadougou, the capital city (Fig. 1). The watershed has an area of 22 km$^2$. The region has Sahelian climate with an average annual rainfall of 400 mm and two seasons: the wet season from June to October and the dry season from November to May (Sivakumar and Gnoumou, 1987).

The boundaries of the watershed are shown in Fig. 1. Sampling occurred from March 24 to 28 2015, during the dry season. Water and soil sampling points were selected such that the transport of pollutants from individual mining sites to the watershed outlet can be determined.

Water samples were collected from a total of 8 sampling points as shown in Fig. 1. W1 was collected from a gold hole at 80 m depth. W2 to W8 were taken at public drinking water taps from groundwater sources. W2 is nearest to the cyanidation site whereas W8 is nearest to the watershed outlet. No surface water was collected because the streams within the watershed were completely dry during the sampling period. Water samples were collected in sterilized non-transparent 500 mL glass bottles. Each sample was preserved by the addition of NaOH pellets according to Standard Methods SM-4500-CN-F (American Public Health Association, 1998) and immediately stored into insulated containers at 4 °C to prevent F-CN transformations during samples transport. Upon arrival at the laboratory, the samples were refrigerated and analysed within 48 h.

Soil samples were collected from 30 sampling points (S1 to S30) at 20 cm depth from the surface. S5, S6, S7 and S1 are cyanidation sites that at the time of sampling were abandoned for 15 d, 1 year, 2 years and 9 years, respectively. Soil samples were collected into plastic bags without the addition of preservatives and immediately stored in insulated containers at 4 °C.

It was assumed that the presence of F-CN in the soil samples at 20 cm depth would be due to the infiltration of surface runoff into the soil and the presence of F-CN in the groundwater samples