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Microcalorimetry and enzyme activity to determine the effect of nickel and sodium butyl xanthate on soil microbial community

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ABSTRACT

In non-ferrous metal tailings, combined pollution in the surrounding soil is caused by heavy metals and flotation chemicals. The combined effects of nickel (Ni) and its primary ore processing collector, sodium butyl xanthate (SBX), on soil microbial activity were investigated following the fluorescein diacetate hydrolase (FDA) and sucrase (SA) activities, and isothermal microcalorimetry during 60 days. FDA and SA activities as well as overall soil microbial activity were significantly affected by Ni, SBX and Ni/SBX mixture. The inhibition rate (I) of the growth rate constant (k) being higher with the Ni/SBX mixture than with SBX alone during the experiment. The growth rate constant (k) was positively correlated ($p < 0.05$ or $p < 0.01$) with enzyme activities (FDA and SA) indicating that k represented a valuable proxy to evaluate the toxic effect of metals and flotation reagents on soil microorganisms. Thus, microcalorimetry was a useful method to characterize soil microbial communities.

1. Introduction

The pollutants produced during ore processing in mining areas can cause severe contamination on the surrounding environment (Li et al., 2014; Wu et al., 2011). In China, a large amount of heavy metals is left in the mine tailings because the mining deposits are poly-metallic and poor. It has been reported that 50–300 g of flotation chemicals are consumed per ton of ore in order to separate and concentrate ores (Fu et al., 2015). Most of flotation reagents are directly discharged into tailings dams leading to the formation of combined pollution in the surrounding environment (Araujo et al., 2010; Lin et al., 2016; Shen et al., 2005).

Sodium butyl xanthate (SBX) is one of the most commonly used reagents in the flotation process of metal ores, particularly for Ni production. Flotation reagents have significant toxic effects on aquatic animals and soil microbial activity (Bararunyeretse et al., 2016; Guo et al., 2016; Loon and Madgwick, 1995). In the past few decades, Ni has become a serious problem as its concentration can reach 26,000 mg/kg in contaminated soil, which is far higher than the environmental standard (Rinklebe and Shaheen, 2017). According to China's soil quality guidelines, commercial and industrial land Ni concentration is limited to 200 mg/kg (GB15618-1995). Like other trace elements, excessive nickel concentrations in the soil may accumulate in plants or livestock and may be transferred to the human body through the food chain (Antoniadis et al., 2017; Thakali et al., 2006; Warren et al., 2003) leading to various acute or chronic diseases (Hartley and Lepp, 2008).

The pollutants produced during the ore processing continuously pollute the soil around the mining area. Microorganisms play a crucial role in soil ensuring soil quality, fertility and detoxification capacities (Gadd, 2010; Green et al., 2006; Rong et al., 2007; Schnürer and Rosswall, 1982). Soil microbiological parameters such as enzyme activities, microbial community structure, and microbial biomass have been proposed as bio-indicators to report soil quality (Dick, 1994; Kakkar and Jaffery, 2005; Lu et al., 2013; Xian et al., 2015). Soil enzymes are known as sensitive indicators of stress (Zhang et al., 2012). Enzyme activity is widely used as a fast and simple method for soil

Abbreviations: Ni, Nickel; SBX, Sodium Butyl Xanthate; TOC, Total Organic Carbon; FDA, Fluorescein Diacetate Hydrolase; SA, Sucrase; N, Nitrogen; K, Potassium; P, Phosphorus

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microbial activity assessment. Among them, the levels of fluorescein diacetate hydrolase (FDA) are related to total enzyme activity (Schnürer and Rosswall, 1982; Stubberfield and Shaw, 1990). Sucrase (SA) not only participates in the direct metabolism of soil organic matter, but also plays an important role in enhancing soil soluble nutrients (Ge et al., 2011).

Compared with enzyme activity, isothermal microcalorimetry is a sensitive, non-invasive and non-destructive method that can monitor live system activity in real time and continuously, provide qualitative and quantitative indicators of soil state and soil destruction (Barros et al., 2007; Bravo et al., 2011; Chen et al., 2009; Yan et al., 1999). Recent studies have focused on the acute toxicological effects of flotation agents and heavy metals on soil microbial activity (Bararunyeretse et al., 2017, 2016; Guo et al., 2016). However, flotation reagents and heavy metals can contaminate the soil environment around the mine, so more analysis is required to determine the toxicity of the co-contaminants made by heavy metal and its corresponding mineral collector (Bararunyeretse et al., 2017).

Considering the above situation, and because the microbial community and activity test can be used as an ideal biological indicator of soil health and pollution, thus the present study was designed. This study involved the toxicological effects of Ni, SBX and Ni/SBX mixtures on soil microorganisms. Combined with microcalorimetry and enzyme activity method, the changes of soil microbial activity at different times were studied to investigate the effects of single pollutants and combined pollutants on soil microorganisms. The relevant results of the study are expected to provide effective information for comprehensive management of non-ferrous metal mining areas and provide scientific basis for pollution prevention and control.

2. Materials and methods

2.1. Reagents

In this study, Ni^{2+} was derived from $NiCl_2{\cdot}6H_2O$. Flotation reagent (SBX) was collected from Beijing Institute of Mining and Metallurgy. For Ni^{2+} and flotation reagents, 10 mg/mL stock solutions were prepared in distilled water and kept at 4 °C.

2.2. Soil sampling and experimental set-up

Soil samples were collected in a farmland in Beijing. After removing the surface layer, the soil was collected at a depth of 2–10 cm and stored in a clean polyethylene bag. Then it was air-dried for 15 days, well mixed and sieved with a 2-mm sieve to remove root fragments, soil macro fauna and large particles. The soil was stored in a polyethylene bag at 4 °C before use.

For toxicity analysis, a fixed dose of Ni (0 and 200 mg/kg soil) or different doses of sodium butyl xanthate (50, 150 and 300 mg/kg soil) was added to 1 kg of soil. For the analysis of the Ni/SBX mixture toxicity, soil samples (1 kg) were incorporated into a fixed dose of Ni (200 mg/kg soil) and different doses of sodium butyl xanthate (50, 150 and 300 mg/kg soil), as shown in Table 1. All soils were incubated at 28 °C and conducted in triplicate. Sub-samples were taken after 1, 7, 14, 28 and 60 days of incubation for further analysis.

Soil pH was determined with a soil to water ratio of 1:2.5 (Zhu et al., 2018). Total organic content (TOC) of soil was measured using a TOC meter (Shimadzu, Japan). Nitrogen (N) was analyzed by elemental analyzer (VARIO EL3, Germany). Potassium (K) was determined by flame photometry using the previously obtained calibration curve and Phosphorus (P) was determined by spectrophotometry. Total Ni was measured by inductively coupled plasma mass spectrometry ICP-OES (Thermo iCAP 7000 SERIES, USA) after digestion with nitric acid and perchloric acid (Guo et al., 2016).

The soil used in this experiment contained originally 15.2 g/kg of total organic content, 64.2 mg/kg of total N, 69.8 mg/kg of P, 33.63 mg/kg of Ni, and 123.4 mg/kg of available K. Soil pH was 7.65.

Table 1
Contaminant concentrations for each treatment.

Contaminant	Treatments (mg/kg)							
	Control	S1	S2	S3	S4	S5	S6	S7
Ni	0	200	0	0	0	200	200	200
SBX	0	0	50	150	300	50	150	300

2.3. Analytical procedure: microcalorimetric analysis

Microcalorimetry can directly measure microbial activity and record continuous heat information, providing qualitative and accurate quantitative information on metabolic processes. The exponential phase of the power-time curve is consistent with the exponent of cell density. We used the isothermal multi-channel microcalorimeter TAM IV (TA Instruments, Delaware, USA) to measure soil microbial heat. In short, soil samples were first regulated at microcalorimetric temperatures (28 °C) (Chen et al., 2009). Each sample was supplemented with 0.2 mL of a nutrient solution containing 5.0 mg of glucose and 5.0 mg of ammonium sulfate. The mixture of nutrients has been confirmed by previous studies, providing sufficient nutrients (C, N, S) to stimulate soil microbial activity (Wang et al., 2010; Zhou et al., 2009).

For each microcalorimetric profile, the peak power (P_{peak}) and its corresponding peak time (T_{peak}) were obtained from the power-time curve. The total heat (Q_{peak}) was calculated as the area limited by the power-time curve. Semi-logarithmic conversion of heat flow rate data was used as previously reported (Wang et al., 2010) (Eq. (1)).

$$Pt = P_0 \quad exp(kt) or InP_0 = InP_0 + kt \tag{1}$$

The growth rate constant (k) of soil microorganisms under each poison treatment was determined by a linear fit equation, while the value of the inhibitory ratio (I) was calculated on the values of k (Eq. (2)).

$$I(\%) = \frac{k_0 - K_c}{k_0} \times 100 \tag{2}$$

where P_0 and P_t are the heat output at time 0 and time t, respectively. I (%) is the inhibition rate, k_0 and Kc are the growth rate constant of the control and the growth rate constant of soil microbes under the corresponding toxicant, respectively.

The growth rate constant (k) and inhibition rate (I) are important thermodynamic parameters for microbial metabolism and they have been widely used as quantitative assessment methods for the toxic effects of various chemicals on soil microbial activity (Nunezregueira et al., 2006; Wang et al., 2010; Zhu et al., 2018).

2.4. Determination of soil enzyme activities

The level of fluorescein diacetate hydrolyze (FDA) activity correlates with the overall enzyme activity (Schnürer and Rosswall, 1982). For FDA determination, 0.1 mL of fluorescein diacetate stock solution (1 mg/mL) and 5 mL of potassium phosphate buffer (pH 7.6) were added to 1 g of wet soil. After the samples were mixed by vortex, they were shaken in the dark at 37 °C in a water bath for 20 min. Then 5 mL acetone was added and mixed. To separate the supernatant from the mixture, the tube was centrifuged at 2000 rpm for 10 min. Absorbance was measured at 490 nm (Adam and Duncan, 2001). The fluorescein stock solution was used to make the fluorescein calibration curve.

Sucrase (SA) activity is not only involved in the direct metabolism of soil organic matter, but also plays an important role in enhancing soil soluble nutrients (Ge et al., 2011). Five grams of dry soil (28 °C for 1 day), 15 mL 8% sucrose solution, 5 mL phosphate buffered to pH 5.5,