



Applications of Cr-rich composted tannery sludge in the soil decrease microbial biomass and select specific bacterial groups

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Abstract

The tannery industries generate a solid waste known as tannery sludge, which is composed of organic and inorganic compounds, mainly chromium (Cr). When Cr is not removed from the tannery sludge, this solid waste is metal-rich and its application could affect the soil microorganisms. Alternatively, the composting of the tannery sludge can contribute to decreasing the concentration of Cr in the composted tannery sludge (CTS). However, in some cases, the concentration of Cr remains high in the CTS. During the last 10 years, the Cr-rich CTS has been successively applied in the soil, and its effect on soil microbial properties was verified. Here, we discuss the effect of successive applications of Cr-rich CTS on soil microbes. Interestingly, the findings have shown that successive applications of Cr-rich CTS selected specific soil microbial groups with potential functions. In addition, the studies added a new focus to further research evaluating the potential effect of successive applications of Cr-rich CTS on the rare microbial community.

Keywords Environmental science · Waste management · Soil microbiology · eDNA sequencing

Introduction

World industrialization has promoted a significant generation of solid waste, which is increasing over the years, being estimated at a total of ~3.9 billion tons of solid waste around 2050 (Kaza et al. 2018). Therefore, strategies to recycle these solid wastes have been reinforced worldwide, mainly in the developing countries. The recycling of solid wastes brings several benefits, from the reduction of solid wastes being discarded into the environment to their reuse in the economic process, such as agricultural production. Among the main solid wastes generated by industries, the tannery sludge, a solid waste produced by tannery industries, presents several organic and inorganic compounds, such as

organic C, N, P, and Ca, which could contribute to agriculture. On the other hand, tannery wastes usually present salts, and their application in soils can increase the soil salinity (Alvarez-Bernal et al. 2006). Particularly, the tannery sludge presents a high content of chromium (Cr) that is used during the tanning process. Interestingly, the removal of Cr found in tannery wastewater can be done by chemical precipitation methods (Mella et al. 2015). Therefore, this metal can be reused in the tannery industry during the tanning process (Ahmed et al. 2016). Consequently, it contributes to maintaining the tannery sludge with low content of Cr, being safe to be discarded in the environment.

However, when processes of Cr removal are not well applied, the tannery sludge can present high content of Cr that prevents its application in agricultural soils. Thus, the application of Cr-rich tannery sludge could contaminate the soil and affect negatively the soil microorganisms, decreasing the microbial biomass, with consequences on soil enzymatic activity (Babich et al. 1982). To prevent potential contamination by the application of Cr-rich tannery sludge in the soil, alternative methods for tannery sludge recycling have been recommended aiming to improve this waste. Among the alternatives to recycling solid wastes is the use of composting, a process that has been applied for years in Cr-rich tannery sludge (Araujo et al. 2020). The main

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objective in applying composting as a method to recycle tannery sludge is the potential to biodegrade Cr present in this waste. However, it is not clear if the compost obtained from tannery sludge is safe to be applied to the soil, mainly considering its potential effect on soil microbial properties.

The potential of composting in the biodegradation and recycling of Cr-rich tannery sludge

Composting is a biological alternative to aerobically decompose organic wastes, occurring mostly under thermophilic conditions, and aims to eliminate toxicity, pathogens, and weed seeds, obtaining a stabilized material (Petruccioli et al. 2011). Indeed, it is well known that composting is effective to biodegrade and recycle organic wastes, decreasing their potential toxicity (Ayilara et al. 2020). This biological process has been indicated to recycle several types of organic wastes, such as cattle manure, sewage sludge, and industrial wastes (Araújo and Monteiro 2006; Moretti et al. 2015), being recommended to improve organic wastes before they can be applied in the soil. However, the potential of composting in the biodegradation and recycling of metal-rich organic wastes and whether this process is effective in detoxifying these wastes, mainly decreasing the content of metals, are unclear. These issues are important since high concentrations of metals in soils may be toxic to soil microbes (Okereafor et al. 2020).

Therefore, based on the assumption that composting is effective in detoxifying organic wastes, this process has been applied to biodegrade Cr-rich tannery sludge and then to verify the effect of the composted tannery sludge (CTS) application on soil microbes (Santos et al. 2011; Silva et al. 2014; Araujo et al. 2020). Although Barthod et al. (2018) reported that composting is not effective to biodegrade metals, previous studies showed that composting is effective in degrading the Cr-rich tannery sludge, resulting in a significant decrease of Cr content in the CTS (Santos et al. 2011; Silva et al. 2014; Araujo et al. 2020). Thus, the Cr-rich tannery sludge was mixed with raw materials (sugarcane straw and cattle manure) and submitted to composting by an aerated pile method for 90 days (USDA 1980). The obtained CTS was analyzed, and the results showed a decrease in the Cr content (Santos et al. 2011), but still 2-fold higher than the upper limits for Cr in the Brazilian regulation (CONAMA 2006). Thence, this compost was classified as a Cr-rich CTS. A possible explanation for the reduction of Cr concentrations after the composting process refers to the changes that occurred by the reduction/oxidation processes, to a less available and more stable molecular form or the total breakdown of the metal and its reduction in concentration as a chemical element. Another explanation would be

due to a dilution process instead of biodegradation (Santos et al. 2011), as also reported in previous studies assessing composting of solid wastes (Amir et al. 2005; Moretti et al. 2015; Zhang et al. 2017). Haroun et al. (2009) composted tannery sludge for 60 days and observed a decrease in Cr content, and they attributed it to metal loss through leaching during the composting process, mainly in the thermophilic phase, being associated with Cr released from decomposed organic matter.

Effect of Cr-rich composted tannery sludge on soil microbial biomass

Since the compost obtained by Cr-rich tannery sludge remains with a high concentration of Cr, some studies were conducted to assess the effect of applying this compost on soil microbial biomass, in both laboratory and field conditions (Santos et al. 2011; Silva et al. 2014; de Sousa et al. 2017; Araujo et al. 2020). The decision to use soil microbial biomass (SMB) to assess the effect of Cr-rich CTS is because SMB represents the living component of the soil organic matter (SOM) (Jenkinson and Ladd 1981). SMB is considered an early and sensitive indicator of soil contamination and can be used to predict long-term trends in soil quality (Ulea et al. 2017), being used as a sensitive microbial indicator to assess the effect of successive applications of organic wastes, such as municipal waste (Bastida et al. 2008; Walia and Goyal 2010; Srivastava et al. 2016). However, for the first time, SMB has been used to assess the potential effect of successive applications of Cr-rich CTS on soil microbial properties.

The successive application of Cr-rich CTS for more than 10 years resulted in a decrease in SMB content (microbial C and N), mainly when this compost was applied at higher rates (e.g., 10 and 20 ton ha⁻¹) (Araujo et al. 2020; Fig. 1). Interestingly, during the first years of application, SMB increased at the lowest CTS rate (2.5 t ha⁻¹), probably due to the addition of available C and N sources that stimulated the soil microorganisms. However, the successive application of Cr-rich CTS increased the content of Cr in soil, varying from ~11 (2.5 ton ha⁻¹ CTS) to ~135 mg kg⁻¹ (20 ton ha⁻¹ CTS) after 10 years of applications. These increased Cr concentrations negatively affected SMB (Araujo et al. 2020). It occurred because high concentrations of metals damage cell membranes and DNA, alter enzymes, and disrupt the cellular functions of microbes (Bruins et al. 2000). On the other hand, SMB has limitations to indicate the potential effect of metal-rich wastes since microbial biomass is a measurement of the total microorganisms, in terms of biomass (Brookes 2001), and does not discriminate who are the main microbial groups. Therefore, it is necessary to assess

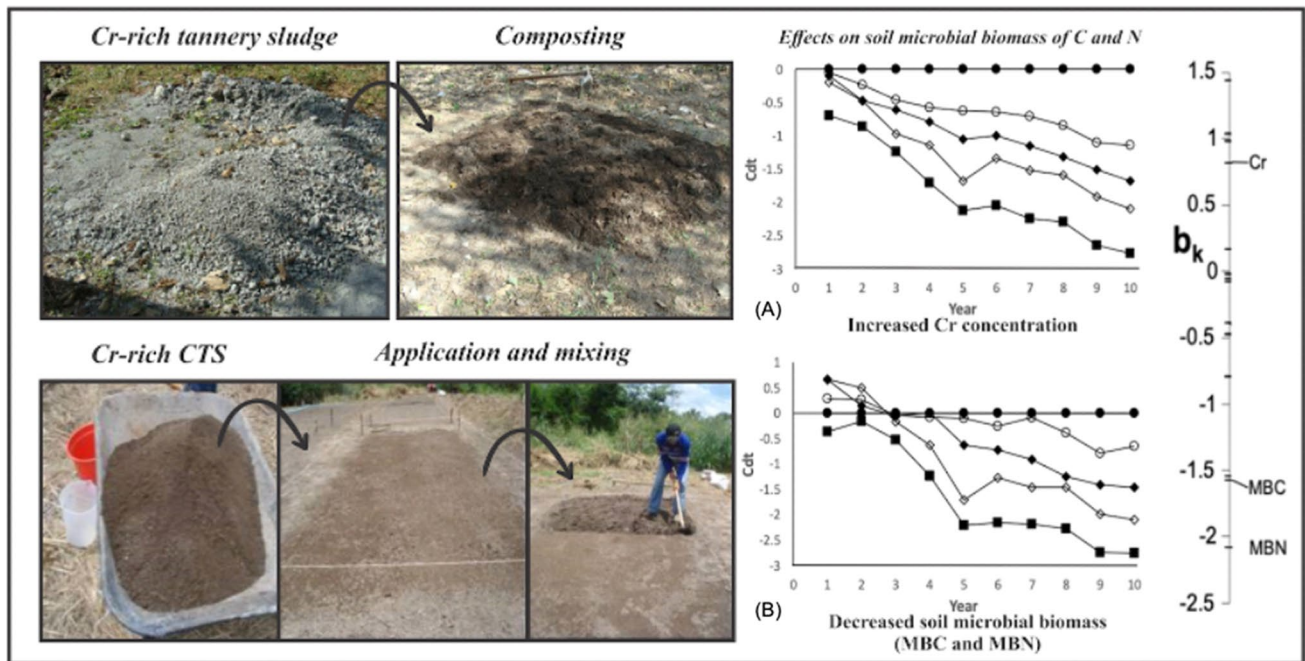


Fig. 1 Composting and application of Cr-rich CTS in the soil. Accumulation of Cr (A) and responses of soil microbial biomass (B). The lines represent the course of the treatment levels in time. The species weight (b_k) can be interpreted as the affinity of the properties with the

principal response curves (c_{dt}). Positive (b_k) means an inverse pattern of curve; i.e., Cr concentration increases over years. In contrast, negative (b_k) means a direct pattern of curve; i.e., soil microbial biomass decreases over years (adapted from Araujo et al. 2020)

the potential effect of successive applications of Cr-rich CTS on soil microbial groups in a more discriminate approach.

Successive applications of Cr-rich composted tannery sludge changes bacterial diversity and select specific bacterial groups

Soil microbial communities occupy the most biologically diverse habitats in the world, being composed by different groups (e.g., bacterial, fungal, and archaeal groups) that provide crucial ecosystem functions and services (Falkowski and Tom 2008). Interestingly, soil microorganisms are highly sensitive to disturbances, being considered bioindicators to assess the potential effects of solid wastes on the soil environment, especially those rich in metals.

The analysis of the soil microbial groups can be done by cultivation-dependent and independent methods (Salmonová and Bunešová 2017). Cultivation-dependent methods are based on the inoculation and incubation of microbes under growth media. Positively, these methods allow the growth and purification of single microorganisms, which provides their morphological, physiological, biochemical, and molecular characterization (Mandal et al. 2011). As a limitation, the cultivation of a particular microbial group needs specific types of nutrients and selective antibiotic agents (Vlková et al. 2015).

Currently, cultivation-independent methods, such as those based on DNA sequencing from environmental samples, have allowed for monitoring the status of microbial groups in soils from a wide range of environments (Araujo et al. 2022; Mendes et al. 2017). Interestingly, the sequencing of the 16S rRNA gene from soil samples presents high efficiency and potential to show the relationship between microbial communities and their potential functions with environmental drivers, such as metal concentration (Jones et al. 2021). Therefore, previous studies have reported alterations in the microbial communities after the application of metal-rich solid wastes (Oijagbe et al. 2018). On the other hand, it can select specific microbial groups, some of them classified as rare species, which present interesting potential functions.

Since the measurement of soil microbial biomass can limit a clear understanding of the effect of soil Cr-rich CTS application on specific microbial groups, DNA sequencing has been applied in soil samples submitted to successive applications of Cr-rich CTS. Therefore, Miranda et al. (2018a) assessed the bacterial community in soils after 7 years of application of Cr-rich CTS and found an increased abundance of specific bacterial groups, such as *Bacillus*, *Paenibacillus*, *Symbiobacterium*, *Clostridium*, *Microlunatus*, and *Actinomadura*. The increased abundance of some specific microbial groups may be interesting as they can assist plant growth. For instance, *Bacillus*, *Paenibacillus*,

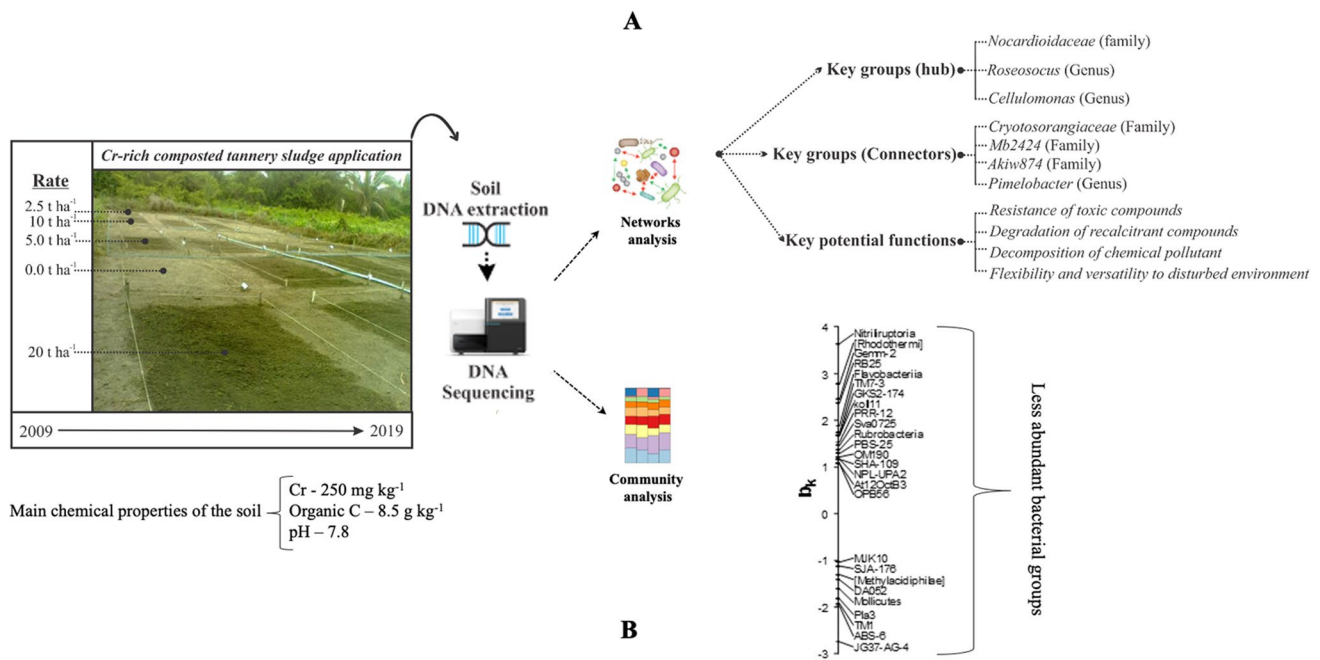


Fig. 2 Effect of successive application of Cr-rich CTS (> 10 years) on soil bacterial community. **A** Effect of high rates (10 and 20 t ha⁻¹) selecting specific microbial groups with potential functional-

ity (Ishimoto et al. 2021). **B** Diagram of bacterial classes indicating the effects of Cr-rich CTS into the soil (extracted from Ishimoto et al. 2021; Miranda et al. 2018a)

and *Clostridium* are well-known plant growth promoters (Hussain et al. 2020). Regarding the archaeal community, the application of the Cr-rich CTS increased the abundance of Thaumarchaeota (Miranda et al. 2019), which presents the capability to oxidate ammonia, acting on important steps of the nitrogen cycle (Holmes et al. 2019). Interestingly, these studies have shown a decrease in the community variability after successive application of the Cr-rich CTS, suggesting a potential environmental filter (probably the high Cr concentration), which could select specific microbial groups due to the soil disturbance (Fraterrigo and Rusak 2008). Thus, the application of the Cr-rich CTS would promote selective pressure on microbial communities (Miranda et al. 2018b).

Considering that the successive application of Cr-rich CTS has promoted a possible selection of specific microbial groups, Ishimoto et al. (2021) applied a co-occurrence network analysis to verify the microbial dynamics and identify key microbes in soil samples with the application of Cr-rich CTS. Firstly, this study identified that Proteobacteria and Actinobacteria are the most interacting bacterial groups in the soil under Cr-rich CTS. Although both microbial phyla present decomposers and plant growth promoters, the key microbes detected belonged to the Actinobacteria phylum found in the highest Cr-rich CTS rate and consisted of active degraders of recalcitrant and pollutant compounds (Ishimoto et al. 2021). This result suggests that a successive application of Cr-rich CTS, at high rates, contributes to selecting specific microbial groups with potential functionality to

resist a high concentration of toxic compounds and activity recalcitrant chemical degradation (Figure 2A). For instance, the family *Nocardioideaceae*, found at the highest rates of compost, presents a high capability to degrade complex compounds, including toxic environmental pollutants, such as metals (Tóth and Borsodi 2014).

Finally, the studies assessing the effect of Cr-rich CTS in soils revealed that the successive applications promoted changes in the less abundant microbial groups. Indeed, Miranda et al. (2018a) applied the principal response curve method to assess the effect of different Cr-rich CTS rates on bacterial groups and found some less abundant bacterial groups being driven by the compost (Figure 2B). This finding adds a new focus to further research in assessing the effect of successive applications of Cr-rich CTS on the rare biosphere.

Concluding remarks

This short essay discussed the most interesting results obtained from a field experiment with successive applications of a Cr-rich CTS for years. The main finding is that the applications of Cr-rich CTS during 10 years promoted a selection of specific microbial groups with distinct functions. Interestingly, the application of the highest rates of compost selects microbial groups related to the degradation of recalcitrant and pollutant compounds, which brings the

perspective of potential microorganisms to be used in bioremediation purposes. In addition, further studies focusing on rare microbes can clarify the patterns of the rare biosphere in Cr-contaminated soils.

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Data availability The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate Not applicable.

Consent to publish Not applicable.

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