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Short communication

Effect of crushed mussel shell addition on bacterial growth in acid polluted soils

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ABSTRACT

We applied three different doses of crushed mussel shell (CMS) on two Cu-polluted acid soils to study the effect of these amendments on the growth of the bacterial community during 730 days. Soil pH increased in the short and medium term due to CMS addition. In a first stage, bacterial growth was lower in the CMS-amended than in the un-amended samples. Thereafter, bacterial growth increased slowly. The soil having the highest initial pH value (4.5) showed the first significant increase in bacterial growth 95 days after the CMS amendment. However, in the soil with the lowest initial pH value (3.8) bacterial growth increased significantly only after 730 days from the CMS addition. The highest dose of CMS caused that, at the end of the incubation period, pH value have increased 2 units, whereas bacterial growth was 4–10 times higher. In view of these results, CMS amendment could be considered as an agronomic sound practice for strongly acid soils (pH <4.5).

1. Introduction

Heavy metal contamination is one of the most important environmental concerns regarding soils and mine tailings. Some human activities, such as mining and intensive agriculture, lead to elevated metal concentrations in soil (Kabata-Pendias and Pendias, 2001). Agriculture has promoted heavy metal accumulation in soils due to inorganic and organic fertilization, liming, sewage sludge amendments, irrigation waters and pesticide applications (Nagajyoti et al., 2010). Cu is widespread, and it is a very relevant toxic pollutant, particularly in vineyards soils where the use of Cu-based fungicides lead to high increases of Cu levels (Komárek et al., 2010). In some cases, these increases in Cu levels take place in strongly acid soils (Fernández-Calviño et al., 2009a; Soler-Rovira et al., 2013). Mine tailings usually contain high concentrations of metals (Cu, Zn, Fe, Ni, Pb and Cd), which can become more mobile and toxic for soil organisms and plants in the low pH conditions created by acid mine drainage (Conesa et al., 2006; Rodríguez et al., 2009). In some Cu-exploited mine tailings, high Cu levels can be detected, with soils presenting very high acidity (Álvarez et al., 2011).

Soil microorganisms, the main agents responsible for long term sustainability of soil ecosystems (Nannipieri et al., 2003), can be significantly affected by both Cu accumulation (Fernández-Calviño et al., 2011a) and changes in soil pH (Fernández-Calviño and Bååth, 2010). Soil pH presents a double role on soil microorganisms, because it can have a direct influence on microbes, and it is often correlated with important environmental factors such as heavy metal availability and toxicity (Degryse et al., 2009). It is well known that heavy metals availability increases when soil pH becomes lower (McBride and Bouldin, 1984). Therefore, recommendable practices to treat metal polluted acid soils are to increase soil pH and/or to add appropriate compounds or materials in order to diminish the metal availability. Organic wastes or by-products and alkaline materials are commonly used to reduce heavy metals availability in acid mine wastes (Pérez-Mora et al., 2005; Alvarenga et al., 2008; Baker et al., 2011). Marble wastes as a source of carbonates were recently tested for the remediation of mining areas (Zornoza et al., 2012, 2013). The use of mussel shell, a waste from seafood industry, can increase soil pH (Garrido-Rodríguez et al., 2013) and reduce heavy metals bioavailability, specifically in the case of Cu, Cd and Pb (Garrido-Rodríguez et al., 2013; Ramírez-

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Pérez et al., 2013). However, the soil microbial response to the addition of mussel shell on acid and contaminated soils has not been tested yet.

One of the most sensitive measurements related with the microorganisms turnover is the bacterial growth (Rousk and Bååth, 2011), which is seriously reduced at low pH (Rousk et al., 2009) and high Cu concentrations (Rajapaksha et al., 2004). Therefore, the addition of mussel shell to acid and contaminated soils could increase bacterial growth by means of promoting pH increases and reduction of heavy metals bioavailability.

In the present work, bacterial growth estimated as Leucine incorporation was determined in two Cu-polluted acid soils for a period of two years. Soil samples were amended with three different crushes mussel shells doses, to be compared with control un-amended samples. We measured the bacterial growth aiming to elucidate the effect of mussel shell addition on the metabolic activity of bacterial communities in very acidic soils contaminated by Cu.

2. Materials and methods

Two contrasting soil samples were taken from the surface layer of the soil (0–20 cm); one of them was sampled in a vineyard area (mean annual temperature 12.7 °C, and mean annual rainfall 1086 mm), and the other one was sampled in a copper mine tailing (mean annual temperature 12.6 °C, and mean annual rainfall 1886 mm). Both soils were air-dried, sieved through 2 mm and stored in polypropylene jars. The crushed mussel shell (CMS) was obtained from Abonomar S.L. (Illa de Arousa, Galicia, Spain) and had a value of 9.4 for pH in water. These soils and the CMS material were used in a previous work (Garrido-Rodríguez et al., 2013), and their main characteristics are shown in Table 1.

Laboratory experiments were performed by adding 6, 12 and 24 g of CMS per kg of soil, corresponding to 12, 24 and 48 Mg ha^{-1} considering an effective soil depth of 20 cm and a soil bulk density of 1 g cm^{-3}. Soil samples without CMS addition was used as control. Two replicates were performed for each dosage. The CMS doses are considered to be appropriate for remedial actions and were used in other studies investigating the potential of CMS for treating these polluted acid soils (Garrido-Rodríguez et al., 2013; Ramírez-Pérez et al., 2013). After dosage, the soil samples and soil + CMS mixtures were rewetted up to 60% of water holding capacity, and then they were incubated at 22 °C. During the period of incubation, the moisture content of the mixtures was checked every 15 days, and the weight of water loss was compensated by adding distilled water. The bacterial growth measurements started 4 days after rewetting, considered as sufficient time for the recovery of bacterial activity (Iovieno and Bååth, 2008), and the measurements were also performed at days 8, 16, 32, 64, 95, 190, 232, 424 and 730 after CMS addition.

The bacterial community growth was estimated using Leucine (Leu) incorporation into bacteria (Kirchman et al., 1985), extracted from soil using the homogenization and centrifugation techniques described by Bååth (1992, 1994) and modified by Bååth et al. (2001). Briefly, soil samples (2 g fresh weight) were mixed with 20 mL distilled water using a multivortex shaker at maximum intensity for 3 min. This was followed by low-speed centrifugation at 1000 × g for 10 min to create a bacterial suspension in the supernatant. Aliquots (1.5 mL) of this suspension were transferred to 2 mL microcentrifugation tubes. Then, 2 μl [3H]Leu (37 MBq ml^{-1} and 5.74 TBq $mmol^{-1}$; Amersham) were added with non-labeled Leu to each tube, resulting in 275 nM Leu in the bacterial suspensions. After incubation for 2 h at 22 °C, growth was finished with 75 μL 100% trichloroacetic acid. Washing and subsequent measurement of radioactivity were performed as described by Bååth et al. (2001).

Table 1
General characteristics of the soils and crushed mussel shell (CMS).

	CMS	Mine soil	Vineyard soil
Sand (%)	99.5	67.4	73.4
Silt (%)	0.3	14.0	12.0
Clay (%)	0.1	18.6	14.6
pH (H_2O)	9.4	3.8	4.5
pH (KCl)	9.0	3.0	3.5
C (%)	12.4	0.3	2.6
N (%)	0.08	0.04	0.17
eCEC (cmol kg^{-1})	30.3	3.9	5.3
Cu_T (mg kg^{-1})	9	651	361

C: total carbon; N: total nitrogen; eCEC: effective cation exchange capacity; Cu_T: total copper.

The distribution of the data was tested for normality by the Kolmogorov–Smirnov (K–S) test. The statistical significance of differences between the controls and amended soils was estimated by parametric statistics (analysis of variance, ANOVA) and Dunnet significant difference tests. The statistical analyses were performed using IBM SPSS statistics 20.

3. Results and discussion

The pH values of the soils and the different mixtures at the beginning and the end of the incubation experiment are shown in Table 2. Soil pH increased in both soils immediately after the CMS addition with all three CMS doses. At time zero, the pH value increased from 4.5 up to 5.4 due to the CMS amendments in the vineyard soil, and from 3.8 up to 4.5 in the mine soil. Considering the whole incubation period, a medium-term effect of the higher doses of CMS (24 and 48 mg kg^{-1}) on pH was also observed in both soils (increase of around 1 pH unit after 730 days of incubation).

The initial bacterial growth, as indicated by ^{3}HLeu incorporation, measured 4 days after rewetting, was 0.90 ± 0.06 pmol Leu h^{-1} g^{-1} for mine soil, and 1.61 ± 0.25 pmol Leu h^{-1} g^{-1} for vineyard soil, both without crushed mussel shell (control samples). After that, these values suffered not marked changes along the experiment, with a general trend to decrease with time. At the end of the experiment (730 days) the bacterial growths were 0.35 ± 0.05 and 1.11 ± 0.06 pmol Leu h^{-1} g^{-1} for the controls in mine soil and vineyard soil respectively. These results are in the range of those reported by Rousk et al. (2009), concretely between 1 and 5 pmol Leu h^{-1} g^{-1} for soils with pH from 4.0 to 4.5. Low bacterial growth values can hinder the recovery of soil functions in polluted soils because the turnover became extremely low. Also, at pH values below 4.5 the overall microbial activity can be affected (Rousk et al., 2009). Likewise, the fact that mine soil bacterial activity showed values 2–3 times lower than that of the vineyard soil can be partly explained by its lower pH values, although other adverse soil conditions, such as lower content of organic matter and nutrients and higher Cu levels, can also be of importance.

The CMS amendments caused overall pH increases and hence higher bacterial growth was expected (Rousk et al., 2009). However, after 4 days, the bacterial growth was not significantly different among control and CMS-amended samples in the mine soil (Fig. 1), and, notably, in the vineyard soil the addition of 24 and 48 Tm ha^{-1} of CMS caused a significant decrease in bacterial growth (Fig. 2). Although high pH values induced by CMS can decrease heavy metal availability, the pH has its own effect on bacterial growth. Both increases and decreases of pH can affect negatively the bacterial growth in the short term (Fernández-Calviño and Bååth, 2010), prior than the bacterial community reaches adaptation to the new pH conditions. After 8 days, the bacterial growth had not yet achieved significant differences among the different CMS treatments in the mine soil, whereas in the vineyard soil the bacterial growth was significantly lower than