Agriculture, Ecosystems and Environment 267 (2018) 165–173

Contents lists available at ScienceDirect

Agriculture, Ecosystems and Environment

journal homepage: www.elsevier.com/locate/agee

Effect of different grain sizes of hydroxyapatite on soil heavy metal bioavailability and microbial community composition

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A R T I C L E I N F O

Keywords:
Hydroxyapatite
Heavy metal
Bioavailability
Microbial community composition

A B S T R A C T

Heavy metal contamination of soils has become a major global environmental problem. Previous studies have reported that hydroxyapatite has been extensively used to immobilize soil heavy metals, and the grain sizes of amendments affect the bioavailability and geochemical stability of heavy metals. However, it is not clear how soil microbial diversity and community composition in these soils vary with the application of hydroxyapatite. A field study was conducted to determine the effect of different grain sizes of hydroxyapatite on Cu and Cd bioavailability and microbial community composition. Results showed that micro-hydroxyapatite (MAP), nano-hydroxyapatite (NAP) and normal hydroxyapatite (AP) increased soil pH and decreased soil exchangeable acid and exchangeable Al. Compared with the control, MAP was the most efficient in decreasing the $CaCl_2$-extractable Cu and Cd by 97.5 and 86.5%, respectively, and exchangeable Cu and Cd by 82.4% and 27.4%, respectively. The soil microbial community composition varied with the amendments of different sizes, and the soil bacterial diversity increased after the amendment applications. Analysis of the network structure showed that fewer operational taxonomic units were enriched or depleted between the MAP- and NAP-amended soils, which explained the similarity between the MAP- and NAP-amended soils. These results suggest that MAP may be the best material for amending heavy metal-contaminated soils when considering immobilization efficiency and soil biological characteristics.

1. Introduction

Heavy metal contamination of soilshas become a major global environmental problem as a result of both anthropogenic and natural activities (Zhao et al., 2014; Hu et al., 2016). Heavy metals, represented mainly by cadmium, lead, arsenic, and mercury, are playing unknown physiological functions in living organisms (Ali et al., 2013; Giller et al., 2009). It is clear that soil heavy metals are very toxic to humans, plants and animals, as well as microbes, even at low concentrations (Bååth et al., 1998; Giller et al., 2009). Many studies have shown that heavy metal contamination of soils can cause a decrease in microbial diversity and changes in the microbial community composition (Giller et al., 2009; Kozdrój and van Elsas, 2001; Wei et al., 2016). However, it is not clear how soil microbial diversity and community composition vary in response to the application of amendments in heavy metal-contaminated soils.

Previous studies have reported that the grain sizes of amendments affect the bioavailability and geochemical stability of soil heavy metals (Dong et al., 2016; Chen et al., 2006; Berber-Mendoza et al., 2006). For example, nano-hydroxyapatite (NAP) is advocated as a promising P nanofertilizer with a low loss risk and increased output efficiency compared with conventional water-soluble P fertilizers, due to its nanoscale size, low leaching rate, and slow P-release kinetics (Liu and Lal, 2014; Montalvo et al., 2015). Chen et al. (2006) found that the rock phosphate with a small grain size ($< 35\,\mu m$) is more effective in decreasing the bioavailability and increasing the geochemical stability of soil metals than those of larger size grains (133–266 μm), possibly due to its higher specific surface area and the more available P for the formation of metal phosphates. However, Shi et al. (2009) noted that the grain size (75–380 μm) of mine tailing did not significantly affect the adsorption capacities for Pb(II), Cr(III), Cu(II), Cd(II) and Ni(II). Our previous study showed that micro-hydroxyapatite (MAP, $< 12\,\mu m$) was more effective in decreasing the availability of Cu and Cd than NAP (< 60 nm), which may be attributed to the aggregation of NAP (Cui

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https://doi.org/10.1016/j.agee.2018.08.017
Received 31 October 2017; Received in revised form 15 August 2018; Accepted 20 August 2018
Available online 01 September 2018

et al., 2013). It may be also related to the different soil microbial activities with different grain sizes. However, we still do not understand how the different grain sizes of amendments influence the heavy metal-contaminated soil microbial diversity and community composition.

It is clear that soil is a biologically, physically, and chemically diverse entity that forms the basic substrate of terrestrial ecosystems (Dominati et al., 2010). Usually, soil microorganisms are more sensitive to heavy-metal stress than plants or soil macrofauna, and the microbiological properties of soil are expected to fulfill their potential as an indicator or index for monitoring soil pollution by heavy metals or other pollutants (Brookes and McGrath, 1984; Zhang et al., 2010). Here, heavy-metal stress mainly refers to their bioavailability, that is, the biologically available chemical fraction (or pool) that can be taken up by an organism and react with its metabolism (Tessier and Turner, 1995). Hence, we suggest that the efficiency of remediation should be evaluated not only by chemical effects, but also biological effects, particularly in terms of microbes. Although some studies have investigated the remediation efficiency (chemical and biological effects) of hydroxyapatite, these investigations were carried out in the laboratory and failed to take into account the effects of hydroxyapatite with different grain sizes on soil microbial community composition. In our previous study, we found that MAP showed a more positive and stronger influence on soil catalase and urease activities than NAP (Cui et al., 2013). Moreover, Wei et al. (2016) found that the soil bacterial diversity and community composition were altered after NAP application in an e-waste-contaminated soil. In particular, the percentage of Stenotrophomonas sp. and Bacteroides increased. Therefore, we suspect that the soil microbial community will show a positive response to MAP amendments and that the community composition may differ from other treatments.

In this study, we systemically investigated the effects of different grain sizes of hydroxyapatite on soil microbial community composition on a field scale. We hypothesized that hydroxyapatite amendments with a smaller grain size are more effective than those with larger grain sizes in decreasing Cu and Cd bioavailability, which in turn increase the soil microbial diversity and alter the microbial community composition.

2. Materials and methods

2.1. Study site and experimental design

The study site is located in Guixi City (28°19′N, 117°12′E), Jiangxi Province, China (Fig. S1), close to a large smelter. It has been contaminated for more than 30 years since heavy metal-contaminated water was used to irrigate the farmland. The main pollutants of the contaminated agricultural field are Cu and Cd (Li et al., 2009). The pH was 4.61 and the soil total carbon and cation exchange capacity content were 18.8 g kg^{-1} and 81.3 mmol kg^{-1}, respectively. The average concentrations of Cu and Cd were 605 mg kg^{-1} and 880 μg kg^{-1}, which were 12 and 2.93 times of the National Soil Environmental Quality Standard II (GB15618-1995), respectively. In 2010, crops and even weeds could not grow in the soil before the experiment. At present, the soil is no longer being polluted by contaminated irrigation water, but still suffers from acid rain, and dust emitted from local enterprises.

The experiment was carried out in a completely random plot design using four treatments with three replicates per treatment in November 2010. The four treatments were micro-hydroxyapatite (MAP), nano-hydroxyapatite (NAP), normal hydroxyapatite (AP), and no amendment (control, CK). Each plot was 2 m × 2 m squares. Each amendment was mixed with the top soil (0–17 cm) in each plot using a harrow. The application rate for all amendments was 25.9 tons ha^{-1} (1.16%, w/w) based on previous studies (Dong et al., 2016; Cui et al., 2013; Wei et al., 2016). The soils were immediately irrigated with tap water (500 tons ha^{-1}). After one week, a compound fertilizer ($N:P_2O_5:K_2O$ = 15:15:15, 0.83 ton ha^{-1}) was applied and ryegrass (0.05 ton ha^{-1}) sown in each plot. Ryegrass accompanied with fertilizers was sown each year from 2011 to 2013, but amendments were only applied in November 2010.

2.2. Materials

MAP and NAP were obtained from Emperor Nano Material Co. Ltd (Nanjing, China) and AP was purchased from Hubei Nanzhang Changbai Mineralization Industry Co., Ltd (Hubei, China). Transmission electron microscopy imaging of the different grain sizes of hydroxyapatite indicated that the shapes of MAP, NAP, and AP were spherical, acicular, and bulk, respectively (Fig. S1). The pH of AP (9.05) was the highest, and MAP (7.72) and NAP (7.17) had similar pH values. Low concentrations of Cu (4.4–9.54 mg kg^{-1}) and Cd (37.1–38.3 μg kg^{-1}) were found in MAP, NAP, and AP, except for 1180 μg kg^{-1} Cd in AP (Table S1). The particle size of the three amendments followed the order NAP < MAP < AP, which was consistent with their specific surface areas.

Ryegrass *Lolium multiflorum* Lam. seeds were purchased from Nanjing Shenzhou Seeds Industry Co., Ltd., Nanjing, China. Ryegrass was selected because it is a suitable species for the revegetation of heavy metal-contaminated soils (Bulbovas et al., 2015). Yellow foxtail *Setaria glauca* (*Setaria pumila* (Poir.) Roem. & Schult. subsp. *pumila*) is a native weed (not intentionally sown) in local farmland soil that grew with the application of alkaline amendments in our preliminary experiment.

2.3. Sample collection

Ryegrass failed to establish in the control soil and only grew in the amended soils in 2011 and 2012. The ryegrass and *S. glauca* in each plot were harvested and removed from the soils during the experiment. However, ryegrass became extinct in 2013 in all treated soils, and only *S. glauca* could grow. The shoots of *S. glauca* were harvested on 5 November 2013. All plant samples were washed with tap water first and then with deionized water. Afterwards, the washed samples were oven dried at 80 °C for 24 h, and ground before analysis. The biomass and heavy metal uptake data for *S. glauca* are provided in the Supporting Information (Table S2).

Afterwards, soil samples (0–17 cm) were collected from three treatments plus the control (12 plots, 3 samples per plot). All samples were sieved to < 2 mm and divided into two subsamples: one subsample was stored at −40 °C for biological analysis, and the other subsample was allowed to dry at room temperature for chemical analysis. A reference soil (RS) was also collected from a nearby paddy field (28°14′ N, 117°05′ E) as a reference (Fig. S1).

2.4. Sample analysis

2.4.1. Chemical analysis

The pH values of the soils and amendments were measured using a 10 g sample mixed with 25 ml of distilled water, which was stirred and left at room temperature (25 °C) for 0.5 h. The pH of the suspension was determined using a pH electrode (E-201-C, Shanghai Truelab Instrument Company, China). The Brunauer–Emmett–Teller (BET) specific surface areas of MAP, NAP, and AP were measured using the single-point BET (N_2) method (Micrometrics Accusorb 2100E, USA). Soil organic matter was analyzed based on Walkley's procedure (Walkley and Black, 1934). Available nitrogen (N) and phosphate (P) of the soil were measured according to Page et al. (1982) and Olsen et al. (1954), respectively. The ammonium acetate (CH_3COONH_4) extractable available potassium (K) was analyzed using a flame spectrometer (Pratt, 1965). Soil cation exchange capacity (CEC) was measured using the ammonium acetate method (Pansu and Gautheyrou, 2006). Soil exchangeable acidity and aluminum (Al) were extracted with 1.0 mol L^{-1} KCl, followed by titration with standard NaOH solution in an N_2 atmosphere (Pansu and Gautheyrou, 2006). The total Cu, Cd, and Zn of the soils and amendments were determined using a flame or