Inorganic phosphorus fertilizer ameliorates maize growth by reducing metal uptake, improving soil enzyme activity and microbial community structure

Wencheng Wu[a,b], Jiahui Wu[b], Xiaowen Liu[b], Xianbin Chen[b], Yingxin Wu[b], Shixiao Yu[a,*]

[a] School of Life Science/State Key Laboratory of Biocontrol, Sun Yat-sen University, Guangzhou 510275, PR China
[b] South China Institute of Environmental Sciences, Ministry of Environmental Protection, Guangzhou 510655, PR China

ABSTRACT

Recently, several studies have showed that both organic and inorganic fertilizers are effective in immobilizing heavy metals at low cost, in comparison to other remediation strategies for heavy metal-contaminated farmlands. A pot trial was conducted in this study to examine the effects of inorganic P fertilizer and organic fertilizer, in single application or in combination, on growth of maize, heavy metal availabilities, enzyme activities, and microbial community structure in metal-contaminated soils from an electronic waste recycling region. Results showed that biomass of maize shoot and root from the inorganic P fertilizer treatments were respectively 17.8 and 10.0 folds higher than the un-amended treatments (CK), while the biomass in the organic fertilizer treatments was only comparable to the CK. In addition, there were decreases of 85.0% in Cd, 74.3% in Pb, 66.3% in Cu, and 91.9% in Zn concentrations in the roots of maize grown in inorganic P fertilizer amended soil. Consistently, urease and catalase activities in the inorganic P fertilizer amended soil were 3.3 and 2.0 times higher than the CK, whereas no enhancement was observed in the organic fertilizer amended soil. Moreover, microbial community structure was improved by the application of inorganic P fertilizer, but not by organic fertilizer; the beneficial microbial groups such as *Kaistobacter* and *Koribacter* were most frequently detected in the inorganic P fertilizer amended soil. The negligible effect from the organic fertilizer might be ascribed to the decreased pH value in soils. The results suggest that the application of inorganic P fertilizer (or in combination with organic fertilizer) might be a promising strategy for the remediation of heavy metals contaminated soils in electronic waste recycling region.

1. Introduction

Soil contamination is a global issue due to the associated risk to the environment and human health. Recently, many studies have reported that the concentrations of heavy metals, especially Cd, Cu, Pb, and Zn were dramatically enriched in farmland soils nearby electronic waste (e-waste) recycling sites (Liu et al., 2015; Luo et al., 2011). Both essential (e.g., Cu and Zn) and non-essential metals (e.g., Cd and Pb) can be toxic to living organisms at excessive concentrations and may adversely affect soil quality, human health, and ecosystems (Kumpiene et al., 2008).

Over the past decades, many technologies such as excavation, washing, and vitrification have been proposed to remedy the metal-contaminated soils. However, these remediation strategies are usually expensive, destructive and result in a considerable amount of waste, which prevents their use in extensively contaminated farmland soils. On the other hand, phytoremediation also seems to be a promising green technology, but it suffers from low metal removal efficiency and negative effects on agricultural productivity (Bolan et al., 2014).

The eco–toxicity and mobility of heavy metals in soil strongly depend on metals' speciation or binding state rather than on total concentration (Singh et al., 2010; Tica et al., 2011; Zhou et al., 2014). One of the most cost–effective remediation strategies for slightly or moderately contaminated soils is metal stabilization by using chemical amendments (Mench et al., 2006). Numerous amendments have been investigated (Bian et al., 2014; Chirakkare and Reddy, 2015; Udeigwe et al., 2011), including novel and traditional materials. The novel materials (e.g., biochar and nanoscale zero–valent iron) have been proven to be effective for immobilizing heavy metals (Mackie et al., 2015; Qiu et al., 2013), however, these materials are very expensive. The traditional chemical amendments such as limestone and calcium hydroxide are effective in reducing metal availability, but their long-term applications can result in adverse impacts on soil quality and microorganisms (Diggelen et al., 2015). Recently, the addition of organic and inorganic fertilizers to immobilize heavy metals has been proposed due to their low cost (Gupta et al., 2014; Singh et al., 2010; Wei et al., 2010) and the improvement of soil nutrient and crop yields (Eo and Park, 2016). However, the

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efficiency of the organic and inorganic fertilizers on immobilizing heavy metals might be different due to their distinct properties, for instance, it has been shown that P fertilizer and organic fertilizer immobilize heavy metals mainly by the process of precipitation with phosphate (Wang et al., 2008), complexation with organic matter (Singh et al., 2010), respectively.

On the other hand, another problem that requires consideration is the assessment of the immobilization efficiency of amendments. The phyto–available portion of heavy metals and its effect on plant growth has been frequently used as a direct indicator in the past (Ciarkowska et al., 2014; Jones et al., 2016; Sevilla-perea et al., 2016). However, these observed changes are generally lagging and not highly sensitive. Recently, parameters related to soil enzymes and microbial community structure have been proposed, because they have been showed as sensitive, early, and effective indicators of soil health (Lu et al., 2015; Pardo et al., 2014; Touceda-Gonz_alez et al., 2015). Nevertheless, few studies have been conducted to evaluate the distinct effects of organic and inorganic fertilizers on heavy metals taking advantage of these soil health indicators.

In the present study, it was hypothesized that (1) both organic and inorganic fertilizers immobilize heavy metals and thus reduce their bioavailability and uptake by crops; (2) both organic and inorganic fertilizers can improve microbial community structure and soil enzymes in heavy metal polluted soils; and (3) organic and inorganic fertilizers would have different remediation effectiveness due to their distinct chemical properties. To verify the hypotheses, a pot trial was conducted to compare the effects of inorganic P fertilizer and organic fertilizer, in single application or in combination, on growth of maize, heavy metal bioavailability, enzyme activities, and microbial community structure in metal–contaminated soils from an electronic waste recycling region.

2. Materials and methods

2.1. Soil and amendments

Topsoils (0–20 cm) were collected from paddy field near an e–waste recycling site located in Longtang Town, which is known as a notorious recycling region in Southern China. Prior to the trial, soils were air–-dried, screened using a 20–mesh stainless steel sieve for chemical analyses, and screened using a 100-mesh stainless steel sieve for heavy metal determination.

Two amendments (calcium–magnesium phosphate fertilizer, PF; commercial organic fertilizer, OF) were tested in single and in combination. For comparison, a treatment with clay mineral (sepiolite, SE) and a treatment without amendment were set as controls. Chemical properties of the tested soil and the amendments are shown in Table 1.

2.2. Pot trial

The soil was ground up to pass through a 4–mm mesh, and then incorporated with the amendment (s). The treatments were set as follows: PF (0.4% w/w), OF (0.4% w/w), PO (0.4% w/w PF + 0.4% w/w OF), SE (0.4% w/w), and CK (non–amendment). All treatments were triplicated. The treated soils were placed into plastic pots (diameter 18 cm × height 30 cm), and each pot contained 5 kg soil. They were equilibrated for six weeks prior to the experiment. Three seeds of maize (Zea mays L.) were planted in each pot and the seedlings were thinned to one plant per pot after germination. During the cultivation, 60% of water–holding capacity of the soil was maintained, and water loss was compensated using tap water (Cd, Cu, Pb, and Zn were not detected).

The maize plants were harvested after 90–day cultivation, and were washed successively with tap water, with deionized water. In addition, the roots were washed with a 0.1 M EDTA solution and with deionized water again. Afterwards, shoots and roots were separated and dried at 60 °C to a constant weight and weighted. The dried plant samples were milled using a stainless steel mill and passed through a 100–mesh sieve for the analyses of heavy metals.

Immediately after harvesting the plants, soil samples were collected from each pot and divided into three subsamples: stored at −80 °C for DNA extraction, sieved to 4 mm for the analyses of soil enzyme activities, and air–dried, grinded and passed through a sieve of 2 mm mesh size and then kept for further analyses of chemical properties and heavy metals.

2.3. Analyses of soil chemical properties

Soil pH was measured after one hour shaking in a soil–distilled water suspension (soil to water ratio of 1:2.5) using a pH meter (model 744, Metrohm, Herisau, Switzerland). Cation exchange capacity (CEC) was determined by the ammonium acetate method after washing with alcohol (Kahr and Madsen, 1995). Total N (TN) was determined with the Kjeldahl method (AOAC, 1990). Total P (TP) was digested with mixed acids and determined colorimetrically. Available P (AP) were determined with the Olsen method (Olsen et al., 1954); and organic matter (OM) was determined by oxidation with a mixture of potassium dichromate and concentrated sulfuric acid and was measured colorimetrically (Schulte, 1995).

2.4. Analyses of heavy metals in soil and plant

The concentrations of heavy metals (i.e., Cu, Pb, and Zn) in soil and plant samples were measured by atomic absorption spectrometry (AAS: PinAAcle 900 T, PerkinElmer, Waltham, MA, USA) after HCl-HNO$_3$-HF-HClO$_4$ digestion, whereas Cd was measured by graphite furnace atomic absorption spectrometry (GF-AAS, PinAAcle 900 T, PerkinElmer) due to the low concentration in samples. The available concentration of heavy metals was quantified after the extraction with diethylene triamine pentacetic acid (DTPA) (Lindsay and Norvell, 1978). Three standard reference materials (two soils: GBW–07430 and GBW–07443; one plant: GBW–10048) from the National Research Center for Certified Reference Materials in China were also analyzed for quality control and quality assurance. The recoveries of the metals ranged from 92.3% to 102.5% (RSD = 3.55%–6.31%, n = 3).

2.5. Soil enzyme and microbial community structure

2.5.1. Soil enzyme activity

Soil urease activity was determined with the method of Tabatabai (1994). Briefly, soil samples were incubated with 10% urea solution at pH 7.1 at 37 °C for 24 h, and then phenol sodium hypochlorite solution was added. After appropriate dilution with Milli-Q water, ammonium was quantified with a spectrophotometer (LengGuang Tech 752sp, CHN) at 578 nm.

Table 1
Chemical properties of the tested soil and the amendments.

Properties	Tested soil	PF	OF	SE
pH	5.00	8.60	7.25	5.44
Cation exchange capacity (CEC, cmol kg^{-1})	20.10	na	na	na
Organic matter (OM, %)	3.99	na	na	na
Total N (TN, g kg^{-1})	2.63	na	na	na
Total K (TK, g kg^{-1})	21.00	na	na	na
Total P (TP, mg kg^{-1})	663.30	na	na	na
Available P (AP, mg kg^{-1})	21.19	na	na	na
Total Cd (mg kg^{-1})	0.58	0.02	0.21	0.01
Total Cu (mg kg^{-1})	264.53	27.98	128.20	32.60
Total Pb (mg kg^{-1})	151.95	52.30	73.33	90.98
Total Zn (mg kg^{-1})	208.63	147.57	268.97	168.83

na: not analyzed.