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Earthworm and organic amendment effects on microbial activities and metal availability in a contaminated soil from China

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ABSTRACT

We tested the effect of an addition of organic matter (OM: 10% of a mixture of coconut bran and cattle dung) and/or earthworm inoculation (15 g fresh weight kg^{-1} soil) in a soil polluted by metals on microbial activities and concentrations of metal DTPA extractable fractions. The experiment, conducted under laboratory conditions, lasted for 60 days. Soil organic C and total N in control were 15.3and 1.47 mg kg^{-1}, and total Zn, Cd, Pb and Cu contents, 405, 0.639, 439 and 394 mg kg^{-1}, respectively. The native earthworm species *Amynthas morrisi* exhibited 91.5% mortality in the un-amended polluted soil, whereas only 20% of *Eisenia fetida* individuals died. In the OM treatment the native species performed much better showing reproduction and a higher soil ingestion rate than *E. fetida*. In both amended and non-amended soils, casts exhibited higher concentrations of total organic C (+15.7 to 46.5%) and N(+13.3 to 59.3%) and alkali hydrolysable N (+78.0 to 133%), but not dissolved organic C. Microbial enzymatic activities were significantly increased when OM was added to the soil (+142 to 456%), with the sole exception of acid phosphatase activity. The addition of earthworms had contrasting effects on microbial activities: *N*-acetyl glucosamine activity was enhanced (+559 to 829%) while no significant difference was noted for other measured enzymatic activities. Introduction of earthworms in OM amended treatments tended to decrease all activities (−30.6 to −59.3%) although they were still higher than in the non- amended soil, especially for β-glucosidase (+182 to 230%). We noted no significant differences between the effects of the two earthworm species. Increased microbial activities resulting from the addition of organic matter did not substantially alter the availability of Zn, Cd, Pb and Cu assessed by their association with DTPA. Earthworm increased the availability of Zn (up to +31%), Cd (+78 to 193%) and decreased Pb (down to −16.4%) in the non-amended treatment. In the amended treatment earthworms induced an increase in Cd (up to 18.8%), but a decrease in Zn availability. Total Cd available concentrations in the experimental units (in soil and casts when earthworms were present) increased from 36.2% in control soil to up to 46.1% after 60 days depending on treatments, while no significant changes were observed for other metals. This rather important change obtained for Cd in such a short amount of time indicates a possible environmental risk.

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1. Introduction

In China, 19.4% of cultivated land is polluted, with 82.8% of this pollution originating from metals contained in contaminated waste, irrigation with contaminated water, chemical fertilizers or non-point source industrial contamination (Meng, 2014). Nevertheless, most of these soils are still used for cropping (Gong et al., 2005). Metal contamination has resulted in degradation of soil quality, decreases in crop yields, and threats to human health (Liu et al., 2005). Remediation of heavy metal polluted soils has therefore become an urgent necessity. Current remediation operations are generally associated with civil-engineering techniques or some environmentally friendly options using phytoremediation (Vangronsveld and Cunningham, 1998; Lasat, 2000; Wong, 2003). A new generation of remediation techniques proposes the

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use of organic matter and earthworm additions to polluted soils. Organic residues are used widely as soil amendments (Rees et al., 2001), and earthworm are expected to enhance the ability of plants to tolerate contaminants and possibly accelerate soil detoxification by chelation processes (Sizmur et al., 2011a,b; Jusselme et al., 2013).

Organic matter added to polluted soil undergoes a process of decomposition that proceeds at variable rates according to climate and soil conditions, the quality of organic matter inputs and soil biological activity (Lavelle et al., 1993; Srivastava et al., 2007). In the process, metals may be either released in available forms (extractable with DTPA) (Salati et al., 2010) or neutralized through chemical chelation processes with humic compounds (Soler-Rovira et al., 2010). Invertebrate soil ecosystem engineers, especially earthworms, are known to stimulate soil physical and microbiological processes with implications for organic matter turnover and nutrient release (Lavelle and Spain, 2001). Observation of enhanced metal availability in polluted soils in the presence of earthworms has led researchers to consider them as potential facilitators of phytoremediation by accelerating the transfer of metals from the soil to plants (Jusselme et al., 2013). Recently published results suggest that both organic amendments and earthworms activities may enhance metal availability in soil. Sizmur and Hodson (2009), however, in a review on the effects of earthworms on heavy metal dynamics conclude that still more research is needed to understand the underlying mechanisms. We tested the hypothesis that organic amendments may help earthworms to survive in highly contaminated soils and that the combined effect of organic amendments and earthworms would result in increased concentrations of metal in the soil solution and/or increased neutralization of metals into complex chemical compounds that plants cannot use (chelates).

2. Materials and methods

2.1. General approach

We analyzed in laboratory microcosms the isolated and combined effects of OM additions and earthworm inoculations on soil organic matter decomposition, microbial enzymatic activities and changes in the availability of metal ions. We used a natural soil with high levels of polymetallic contamination. The epigeic European lumbricid compost worm *Eisenia fetida* and the epi-endogeic native Asian megascolecid *Amynthas morrisi* were introduced into the soil with and without organic matter additions. We compared, in casts produced by earthworms and in the non-ingested control soil, parameters of soil organic matter dynamics (total organic C and N, dissolved organic C (DOC), alkali-hydrolysable N contents and C:N ratio), microbial functions assessed by enzyme activities and metal DTPA extraction.

Dissolved organic carbon (DOC), a mixture of soluble polyphenols, simple aliphatic acids, amino acids and sugars is expected to enhance metal availability by binding metals in organic components (Chantigny 2003; Salati et al., 2010). N availability that frequently limits decomposition through stoichiometric imbalances was assessed by measuring the alkali-hydrolysable N fraction, a mixture of inorganic N (NH^{4+}, NO^{3-}) and labile hydrolysed organic N (amino acids, acyl-ammonium and protein). We assessed a number of key enzymatic activities. Fluorescein diacetate activity (FDA) and soil respiration rate evaluated overall microbial activities (Varma and Oelmüller 2007). β-glucosidase (which degrades cellobiose into glucose), *N*-acetyl-β-D-glucosaminidase (which hydrolyzes *N*-acetyl-β-D-glucosamine into amino sugars) and urease (which hydrolyzes urea into carbon dioxide and ammonia) measured key paths in the decomposition process. Acid and alkaline phosphatase activities assessed organic P transformations. Finally, soil metal DTPA extractable contents were used as indicators of metal availability (Lindsay and Norvell, 1978; Lebourg et al., 1996).

2.2. Soil sampling

Soil was collected at a site located 24°30′N and 113°45′E where pollution has occurred for 40 years due to deposition of metal contaminated effluents carried by the Yanghe River from the Dabaoshan opencast mine (Guangdong Province, South China). Soil was collected from the upper 20 cm in three paddy rice fields, homogenized after air-drying and then sieved at 2 mm. This soil had a low pH, fine texture and relatively low concentrations of organic C and total N (Table 1). Total Cu, Zn, Pb and Cd contents were 2.13, 2.02, 1.76 and 7.88 times greater (respectively) than the allowable limits for arable soils in China according to the National Environmental Quality Standard for Soils of China (GB15618-1995) (Table 1).

2.3. Biological materials

Eisenia fetida were collected from a local vermicompost operation and *Amynthas morrisi* from uncontaminated soils in the Guangdong Province (Southern China). All were clitellate adults. The average fresh weights of *E. fetida* were 0.3 (ranging from 0.2 to 0.5 g ind^{-1}) g fresh weight and 45 individuals were added to one kg soil. *Amynthas morrisi* were 0.4 g fresh weight on average (ranging from 0.3 to 0.6 g ind^{-1}) and 34 worms were added to one kg soil.

Coco bran (*Cocos nucifera* L.) and cattle dung were used as organic inputs in the experiment. Organic C and total N contents and C: N ratio were 50.3%, 0.66%, 76.0:1 for coco bran and 15.7%, 0.62%, 25.4:1 for cattle dung, respectively. Material was air-dried, and sieved at 2 mm. A mixture of 80% Coco bran and 20% cattle dung was used in experiment in order to achieve a suitable C:N ratio (27.6:1) for earthworm growth in soil mixed with organic matter. Total contents of Cd, Pb, Cu and Zn were 0.841, 0, 16.2, 123.7 mg kg^{-1} for dung and 0.733, 0, 0, 50.7 mg kg^{-1} for Coco bran respectively, and these concentrations were all lower than the national quality standard for organic fertilizer of China (NY525-2012).

2.4. Experimental design

One kg of soil was placed in 1.5 L pots allocated to 6 different treatments:

3 Non-amended soil (S group)

(1) S: control soil with no organic inputs nor earthworms.
(2) SE: soil inoculated with 15 g fresh weight of *Eisenia fetida*.
(3) SA: soil inoculated with 15 g fresh weight of *Amynthas morrisi*.

Table 1
Main physio-chemical characteristics and total metal contents of the soil samples (Mean ± S.D., n = 3).

	pH	Clay	Organic C	Total N	C:N ratio	Total Zn	Total Cd	Total Pb	Total Cu
		(%)	g kg^{-1}	g kg^{-1}		mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}
Soil	4.18 ± 0.03	33.6 ± 1.02	15.3g ± 0.23	1.47 ± 0.05	10.4 ± 0.22	405 ± 29.0	0.639 ± 0.06	439 ± 8.44	394 ± 10.2

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