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Short-term effects of cadmium and mercury on soil nematode communities in a pot experiment

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Summary

Analysis of soil nematode feeding groups and functional guilds were used as a valuable tool to detect heavy metal pollution. Effects of cadmium (Cd) at 5 mg/kg, mercury (Hg) at 20mg/kg, combined Cd and Hg at 5+20mg/kg on the nematode communities were studied after three months application. Nematodes were collected from soil in rhizosphere of Morning glories (*Pharhiris nil*) which were applied as heavy metal accumulators and were grown in the experimental pots. Both single and combined heavy metals had marked effects on the nematode abundance, life-history strategies and feeding type composition. Bacteriovores and c-p 2 group were found to be the most abundant trophic group and functional guild, respectively. *Acrobeloides* and *Pratylenchus* were the most two abundant genera, decreasing number of them was responsible for the significant difference between control and polluted treatments. Cd-5 and Cd-Hg 5+20 presented lower values of nematode diversity index (H') and evenness index (J') than Hg-20. The combination of Cd and Hg showed lower nematode trophic diversity (TD), in comparison with single Cd or Hg. Conversely, heavy metals addition exhibited no pronounced effect on Maturity index (MI), structural index (SI) and enrichment index (EI). Our results demonstrate that genera composition is a better indicator to short-term heavy metal effects than some common indicator indices and emphasize that deeper assemblage analyses are needed for a correct interpretation of short-term disturbance on soil nematodes.
Keywords: Morning glory; heavy metals; nematodes; trophic group; c-p group

Introduction

Anthropic activities such as mining, manufacturing, transporting and fertilizing have caused heavy metal contamination in urban and agricultural soils. The heavy metal contamination has resulted in severe threats to human and environment health (Chen *et al*., 2005; Xia *et al*., 2004). Cadmium (Cd) and mercury (Hg) are the two most predominant metals in sewage irrigation region of Liaoning province (Li & Tong 2008). How to deal with the toxic pollutants efficiently and safely is becoming one of the most popular environmental-related themes. Phytoremediation has been developed in recent years (Yoon 2007), and has become a new economical method which can reduce some toxic pollutants in an environmentally friendly way (Pilon-Smits, 2005). Morning glory (*Pharhiris nil*) is a remediation plant which absorbs pollutants from soil and accumulates them in stalk, leaf, and root, in this way, contaminant could be reduced through harvesting the plant body. Xu *et al*. (2012) reported that petroleum degrading rate was increased by 13 % after 2 months of planting Morning glory. With respect to metal bioaccumulation, plants accumulated higher amounts of

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metal in root than stem in previous studies (Ali *et al*., 2004), so the toxicity created by heavy metals impacts roots first, which means soil quality and plant-microbe interactions around the rhizosphere can influence the efficiency of metal phytoremediation (Cherian & Oliveira 2005). However, most investigations about accumulating plants were focused on the aboveground parts, the structure and assemblage of soil fauna around rhizosphere are not well known. Nematodes are one of the most abundant groups of soil invertebrates (Fu *et al*., 2000) and occupy a central position in the soil food web. Soil nematode communities can provide unique insights into soil processes and supply useful information about soil environment. Soil nematode as an indicator for heavy metal pollution has been widely studied in recent years. (Zhang *et al*., 2011; Šalamún, 2011; Martinez *et al*., 2018). Most of previous studies took place in an open environment such as mining area, however, under laboratory condition, the characteristics of rhizosphere nematode communities affected by specific concentrations of single and mixed heavy metals are not well studied. The present study investigated the changes of Cd, Hg and Cd+Hg concentrations after 90 days remediation and characterized the nematode community structure and assemblage around rhizosphere of Morning glory. The objectives of this study were: 1) to evaluate soil quality after phytoremediation experiment; 2) to represent the responses of nematodes to different heavy metal treatments; 3) to provide basic data for using soil nematodes to assess the level of phytoremediation. We hypothesize that only small part of heavy metals could be absorbed by plants during short-term remediation, hence the remaining part still has adverse effects on nematode communities, furthermore, the degree of influence may depend on feeding groups and life-history strategies.

Material and Methods

The seeds of Morning glory were planted in non-contaminated garden soil for 7 days before experiment, then, five seedlings were subsequently transplanted into one pot. Each pot (15cm – diam×12cm – -depth) was loaded by 600g soil collected from Dalian Xishan National Forest Park, soil was loamy with 13.1 % clay, 50.4 % silt and 36.5 % sand and was completely mixed. Based on the concentrations of heavy metals obtained from the wastewater irrigation area (max concentration of Cd = 5 mg/kg) (Liao, 1993) and mercury mining area (Hg = 20.4 mg/kg) (Yu *et al*., 2017), the concentrations of heavy metal were selected at Cd-5mg/kg, Hg-20mg/kg and Cd-Hg5+20mg/kg. Heave metal solutions were applied as $Cd(NO_3)_2{\cdot}4H_2O$ and $HgCl_2$, three replicates at each level were established, concentrations of heavy metals before and after treatment were shown in Table 1. The seedlings were grown for three months in a climate chamber at 20±1 °C. Intensity of light was 5000LX with 16 hour of light alternating with 8 hour of darkness. Each pot was watered with 250 ml deionized water every 2 days.

Nematodes were extracted from 100g (fresh weight) soil per pot using elutriate-sieving-flotation and centrifugation method (Barker *et al*., 1985). Extracted nematodes were heat killed at 60 °C, counted and preserved in 4 % formalin aqueous solution (Steinberger & Sarig, 1993). One hundred nematodes randomly selected specimens per sample were identified to genus level using Olympus inverted compound microscope based on stoma and esophageal morphology (Liang *et al*., 2001, 2003). The genus was identified according to Bongers (1988) and Li *et al*. (2017).

Soil moisture of each sample was gravimetric determined by weight loss at 105 °C for 8 hours and expressed as percent dry weight. Another 100g fresh soil per pot was air-dried for 14 days at room temperature to text soil organic matter and heavy metals. Electronic pH meter (model SevenGo™ pH-SG2), the potassium dichromate external heating method, graphite furnace atomic absorption spectrometry and microwave dissolution/atomic fluorescence spectrometry were used to determined soil pH, organic matter, Cd and Hg, respectively (Ru, 1999; China National Environmental Monitoring Centre,1997; Ministry of Ecology and Environment of the People's Republic of China,2013).

The assemblage and characteristics of nematode community were investigated by following approaches: (1) feeding groups (bacterivores-BF, fungivores-FF, plant parasites-PP, omnivores-predators-

Table 1. Soil chemical properties and heavy metal concentrations.

Heavy metals addition (mg/kg)		Heavy metals contents after remediation (mg/kg)		Average root weight (g/ DW plant)	pH	Soil organic matter (g/kg)
Cd	Hg	Cd	Hg			
0	0	0.21 ± 0.01	0.14 ± 0.01	0.023	6.86 ± 0.27a	40.76 ± 1.95a
5	0	4.12 ± 0.32	0.13 ± 0.01	0.032	6.33 ± 0.21b	42.88 ± 1.54a
0	20	0.21 ± 0.01	18.17 ± 0.67	0.024	6.68 ± 0.30ab	41.31 ± 2.86a
5	20	4.19 ± 0.45	16.38 ± 0.58	0.036	6.42 ± 0.33ab	40.69 ± 3.07a

Heavy metal, root weight and pH data were supplied by Hou (2012); Mean values in a line with different letters are significantly different from each other at P < 0.05; values are expressed as mean ± standard error (n = 3).

OP) (Yeates *et al*., 1993; Pen-Mouratov *et al*., 2004); (2) life-history groups (c–p values range from 1 to 5 represent colonizers to persistors (Bongers, 1990, 1999). (3) ratio of f/b (fungivores/bacterivores); (4) diversity index H'=–∑ni (lnni) where ni is the proportion of individuals in the i-th taxon (Shannon & Weaver, 1949) , (5) trophic diversity index TD = TD = 1/∑pi2, where pi is the proportion of each trophic group; (6) evenness J'= H'/ln(S), where S is the number of taxa; (7) maturity indices MI=∑vifi, where vi is the c–p value of the i-th taxa and fi is the frequency of the i-th taxa in the sample (excluding PP) (Bongers, 1990); (8) enrichment index (EI) and structure index (SI), EI = 100(e/e + b), SI = 100(s/s + b), where e =∑kene, s =∑ksns, and b =∑kbnb (Ferris *et al*., 2001).
All nematode data were 1n (x +1) transformed prior to statistical analysis. The significance of the effects of heavy metals on nematode communities was tested by one-way analysis of variance (ANOVA), SPSS 18.0 statistical software, and means compared by LSD's Test (Least Significant Difference). Differences with P < 0.05 were considered significant. Principal component analysis

Table 2. Relative abundance (%) of nematode genera in control (CK) and polluted pots.

Genus	Guild	CK	Cd-5	Hg-20	Cd5 + Hg20
Panagrolaimus	BF1	0.62 ± 0.61b	0.00 ± 0.00b	5.51 ± 1.14a	5.15 ± 0.67a
Rhabditis	BF1	11.04 ± 0.86a	3.33 ± 3.33b	9.74 ± 1.12ab	7.72 ± 1.92ab
Mesorhabditis	BF1	3.46 ± 1.03a	1.75 ± 1.75a	4.23 ± 0.38a	2.29 ± 1.17a
Acrobeles	BF2	1.68 ± 0.92a	0.00 ± 0.00a	1.28 ± 1.28a	0.00 ± 0.00a
Acrobeloides	BF2	32.30 ± 1.52ab	29.70 ± 2.68ab	27.95 ± 4.56b	42.18 ± 6.71a
Heterocephalobus	BF2	2.89 ± 0.56a	1.75 ± 1.75a	1.67 ± 1.67a	1.01 ± 1.01a
Eucephalobus	BF2	2.27 ± 0.46a	0.00 ± 0.00a	2.56 ± 1.23a	3.57 ± 2.23a
Cervidellus	BF2	0.61 ± 0.61a	0.00 ± 0.00a	1.28 ± 1.28a	1.01 ± 1.01a
Plectus	BF2	0.60 ± 0.60a	0.00 ± 0.00a	1.28 ± 1.28a	1.28 ± 1.28a
Chrohogaster	BF2	0.53 ± 0.53a	0.00 ± 0.00a	0.00 ± 0.00a	1.01 ± 1.01a
Prismatolaimus	BF3	1.19 ± 1.03a	5.46 ± 3.21a	0.00 ± 0.00a	2.29 ± 1.17a
Alaimus	BF4	2.20 ± 1.39b	0.00 ± 0.00b	0.00 ± 0.00b	5.43 ± 1.35a
Paraphelenchus	FF2	0.53 ± 0.53b	0.00 ± 0.00b	0.00 ± 0.00b	5.43 ± 1.35a
Pseudhalenchus	FF2	0.61 ± 0.61a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Aphelenchoides	FF2	8.19 ± 1.78a	6.94 ± 1.53ab	2.95 ± 1.51b	1.01 ± 1.01b
Bursaphelenchus	FF2	0.00 ± 0.00a	0.00 ± 0.00a	2.56 ± 1.28a	1.28 ± 1.28a
Filenchus	FF2	5.79 ± 0.71b	5.27 ± 0.16b	27.56 ± 1.70a	1.01 ± 1.01c
Diphtherophora	FF3	0.53 ± 0.53ab	3.42 ± 1.71a	0.00 ± 0.00b	1.01 ± 1.01ab
Paratylenchus	PP2	17.62 ± 0.52b	33.58 ± 4.33a	8.19 ± 2.05c	10.14 ± 3.30bc
Helicotylenchus	PP3	0.00 ± 0.00b	3.61 ± 1.81a	0.00 ± 0.00b	1.01 ± 1.01ab
Heterodera	PP3	0.53 ± 0.53a	1.67 ± 1.67a	1.28 ± 1.28a	1.01 ± 1.01a
Eudorylaimus	OP4	1.20 ± 0.60a	3.52 ± 1.77a	1.28 ± 1.28a	2.29 ± 1.17a
Aporcelaimellus	OP5	0.61 ± 0.61a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Aporcelaimium	OP5	1.65 ± 0.92ab	0.00 ± 0.00a	2.95 ± 1.51b	2.86 ± 1.61a
Prodorylaimus	OP5	0.61 ± 0.61a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
Discolaimus	OP5	1.14 ± 0.57a	0.00 ± 0.00b	0.00 ± 0.00b	0.00 ± 0.00b

BF: bacterivores, FF: fungivores, PP: plant parasites, OP: omnivores-predators, numbers following the letters in Guild indicate the c-p value of each taxon
Mean values in a line with different letters are significantly different from each other at $P < 0.05$; values are expressed as mean ± standard error ($n = 3$).

Table 3. Ecological indices for nematode community structure in different treatments.

	CK	Cd5	Hg20	Cd+Hg 5+20
H'	2.31 ± 0.21a	1.44 ± 0.14b	1.91 ± 0.20ab	1.68 ± 0.50b
TD	2.34 ± 0.22b	2.85 ± 0.20a	2.42 ± 0.06b	1.78 ± 0.22c
J'	0.85 ± 0.02a	0.70 ± 0.05b	0.84 ± 0.03a	0.70 ± 0.07b
f/b	0.25 ± 0.07b	0.29 ± 0.04b	0.55 ± 0.02a	0.10 ± 0.02c
MI	1.71 ± 0.15a	2.13 ± 0.23a	1.91 ± 0.04a	2.16 ± 0.11a
SI	47.61 ± 7.76a	35.12 ± 17.40a	25.76 ± 6.71a	43.58 ± 0.13a
EI	64.19 ± 4.25a	39.15 ± 15.42a	61.98 ± 1.05a	49.04 ± 15.73a

Mean values in a line with different letters are significantly different from each other at $P < 0.05$; values are expressed as mean ± standard error ($n = 3$).

(PCA) was applied to represent the composition of the soil nematode community using CANOCO software.

Results

Soil chemical properties and heavy metal concentrations

Soil pH dropped dramatically at Cd-5, while soil organic matter increased slightly at Cd-5 and Hg-20. The absorption rates of heavy metals were 17.6 %, 9.15 % and 16.2 %+18.1 % for Cd-5, Hg-20 and Cd+Hg 5+20, respectively (Table1).

Nematode abundance and ecological indices

The average nematode abundance ranged from 150 to 812 individuals per 100g dry soil. Significant difference in the total nematode abundance was found between the control and other treatments (P<0.05) (Table 4). Twenty six nematode genera were identified; of them, 12 genera belonged to the bacteriovores, 4 to fungivores, 4 to plant parasites and 6 to omnivores and predators. *Acrobeloides* was the most abundant genus in all treatments with the highest dominance (42.18 %) at Cd-Hg5+20; *Pratylenchus* dominated the control, Cd-5 and Cd-Hg5+20; *Rhabditis* dominated the control (Table 2). All samples with heavy metal contamination appeared to be dissimilar from the control. The distinction between Cd-5 and CK was most pronounced, whereas the distinction between Hg-20 and Cd-Hg5+20 was not obvious. The abundance of *Panagrolaimus* and *Bursaphelenchus* were positively correlated with Hg and Cd-Hg. The abundance of *Helicotylenchus* was strongly related to Cd (Fig. 1).

Both single and joint toxicity of Cd and Hg to the nematode ecological indices were significant. Cd-5 and Cd-Hg 5+20 reduced the values of diversity index (H') and evenness index (J') (P< 0.05); single Cd heighten the trophic diversity (TD), while the combination of Cd and Hg diminish TD (P< 0.05); Hg increased f/b value, in contrast Cd-Hg reduced the f/b (P< 0.05); heavy metal treatments moderately affected values of MI, SI and EI which fell in relative narrow limits throughout the experiment (P> 0.05) (Table 3).

Nematode assemblage

Bacteriovores were found to be the most abundant group in all treatments, they concentrated at Cd-Hg5+20. Plant parasites were the second most common trophic group with highest proportion at Cd-5. Fungivores were the third most frequent group, their highest relative abundance was found at Hg-20 and the lowest was discovered at Cd-Hg 5+20 (Table 4).

Nematodes classed as c-p 1 and c-p 2 accounted for 87.67 % of all nematodes identified (Table 4). Although the abundance of nematode dropped strongly in polluted soil, the proportion with c-p value 1 and 2 at Hg-20 was much higher than that in the control. Both the highest proportion of c-p3 group and the lowest proportion of c-p 1 group occurred at Cd-5. The relative importance of nematodes with a c-p value of 4 and 5 varied slightly among all treatments.

Discussion

Building on previous researches, population structure that was measured by abundance and genus number (Bakonyi *et al*., 2003), function indexes (MI, SI and EI) (Bongers, 1990; Korthals *et al*., 1996; Wang & McSorley, 2005) and functional variables such as feeding groups (Yeates *et al*., 1993) and c-p groups (Bongers, 1990, 1999) were always applied to analyze the responses of nematode communities to disturbance.

The population of nematodes may increase or decrease with heavy metal concentration (Sánchez-Moreno & Navas, 2007). In the present case the pronounced drop of nematode abundance already sent an alert on the heavy metal pollution (Table 4). Our result is different from previous findings by Bakonyi *et al*. (2003) who reported that Cd had a moderate influence on nematodes. This difference might be contributed by the short-term and high level of pollution. Cadmium could negatively impact on soil enzymatic activities and microbial community structure (Wang *et al*., 2019). We expect that the abundance of nematode was inhibited by the degradation of their feeding sources in contamination soil. In addition, Hg exposure can lead to multitoxicity, and most of these

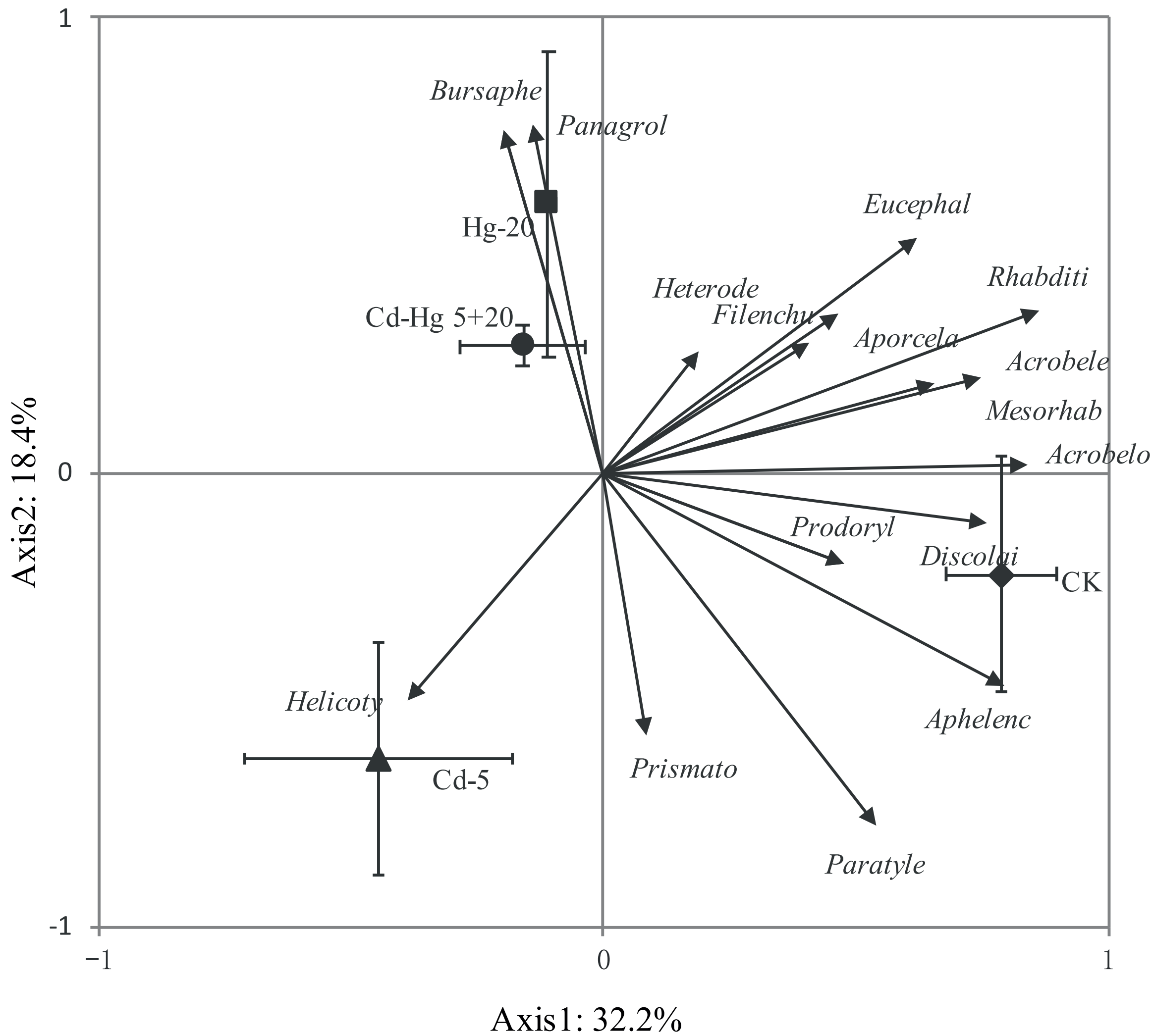

Fig. 1. Principal components analysis (PCA) of nematode abundance in different treatments. Species fit range more than 20% were presented.

harmful effects on nematode can be transferred to progeny (Wu *et al*., 2010). Nematode diversity (H') and evenness (J') were lower at Cd-5 and Cd-Hg 5+20 than the control and Hg-20 (Table 3), which suggested single Cd and mixing of Cd and Hg create worse effects on nematode communities than single Hg. Martinez *et al*. (2019) proposed that nematode-based environmental evaluations should be interpreted in a context-dependent way. Acidic soils can inhibit the diffusion of cadmium (Lu *et al*., 2005) and enhance its bioavailability (Kim *et al*., 2009), but the bioavailability of Hg tends to be lower in acidic soils (Mahbub *et al*., 2016). So the lower values of pH at Cd-5 and Cd-Hg 5+20 seem to be the reason for the lower values of H' and J'. Xie *et al*. (2011) found that the mixing of Cd and Hg had stronger toxic effects on soil microbial community than the single Cd or Hg. Similarly, nematode trophic diversity index (TD) was lowest at Cd-Hg 5+20, which indicated the combination of Cd and Hg exert more adverse impacts on nematode trophic groups than individual Cd or Hg in this study.

Nematode assemblage and function level structure presented by MI, SI and EI was found to be skewed away from the theory of these indices which predicts a reduction of the MI and SI as a consequence of heavy metal pollution. The values of MI, SI and EI were relatively uniform throughout all treatments. Our result was in line with Martinez *et al*. (2018) who discovered a short range for maturity index from different levels of disturbance. The reason for this phenomenon is the rarity and stabilization of relative abundance of high c-p value groups among all treatments. Therefore, we propose that changes in nematode communities could be represented better from abundance and diversity point of view. Since MI, SI and EI are expanded indices derive from proportions of feeding groups and life-history strategies, current findings highlight that more detailed analysis about trophic and c-p groups are needed for a correct interpretation of short-term high level pollution effects on soil nematodes.

Table 4. Absolute abundance (individuals per 100 g dry soil) and relative abundance (%) of nematode guilds in control and polluted pots.

Absolute abundance				
	CK	Cd-5	Hg-20	Cd-Hg 5+20
BF1	120.51 ± 11.68a	8.40 ± 4.84c	30.88 ± 1.56b	23.6 ± 4.43b
BF2	327.48 ± 26.67a	50.04 ± 4.38b	57.36 ± 6.00b	74.68 ± 8.11b
BF3	14.16 ± 8.31a	8.28 ± 4.22ab	0.00 ± 0.00b	3.88 ± 1.49ab
BF4	17.61 ± 11.09a	0.00 ± 0.00b	0.00 ± 0.00b	9.61 ± 2.39a
FF2	121.58 ± 23.30a	19.52 ± 2.79b	52.92 ± 7.46b	14.93 ± 2.96b
FF3	0.00 ± 0.00a	5.52 ± 1.67a	0.00 ± 0.00a	1.84 ± 0.46a
PP2	152.25 ± 5.31a	52.6 ± 4.23b	8.80 ± 2.55c	13.92 ± 2.25c
PP3	4.50 ± 4.50a	4.08 ± 0.10a	2.20 ± 2. 20a	3.72 ± 3.72a
OP4	9.45 ± 4.73a	2.42 ± 1.21b	2.16 ±2.16b	3.68 ± 1.49b
OP5	32.01 ± 8.81a	0.00 ± 0.00b	4.45 ± 2.25b	3.87 ±1.94b
BF	492.05 ± 48.55a	67.61 ± 16.46c	88.21 ± 14.12bc	115.67 ± 43.74b
FF	121.58 ± 23.30a	25.14 ± 7.15c	52.92 ± 7.46b	16.74 ± 6.72c
PP	156.91 ± 9.83a	56.28 ± 4.89b	11.45 ± 3.97c	17.98 ± 6.55c
OP(c-p 4-5)	42.26 ± 13.04a	2.42 ± 1.21b	6.61 ± 2.66b	7.84 ± 3.72b
c-p 1-2	727.47 ± 49.04a	130.48 ± 14.24b	150.12 ± 21.96b	125.92 ± 24.92b
c-p 3-5	85.71 ± 30.07a	20.32 ± 6.31b	8.84 ± 6.68b	27.06 ± 7.40b
Relative abundance (%)				
	CK	Cd-5	Hg-20	Cd-Hg5+20
BF	60.69 ± 4.31b	42.00 ± 3.06c	55.52 ± 0.50b	73.43 ± 5.71a
FF	15.30 ± 3.23b	12.21 ± 2.43b	30.54 ± 1.27a	7.66 ± 1.63c
PP	18.17 ± 1.69b	38.85 ± 7.75a	9.70 ± 1.57b	12.59 ± 4.84b
OP(c-p 4-5)	5.84 ± 1.99a	6.94 ± 2.65a	4.23 ± 0.68a	6.32 ± 2.57a
c-p 1-2	89.53 ± 2.31ab	86.33 ± 3.96b	94.58 ±2.09a	82.10 ± 5.07b
c-p 3-5	11.62 ± 3.33ab	14.67 ± 3.96a	5.42 ± 2.09b	17.90 ± 5.07a

Mean values in a line with different letters are significantly different from each other at $P < 0.05$; values are expressed as mean ± standard error ($n = 3$).
BF: bacterivores, FF: fungivores, PP: plant parasites, OP: omnivores-predators, numbers following the letters in Guild indicate the c-p value of each taxon

Nematode trophic structure

Distribution of soil nematodes within four trophic groups reflects their food-web relations and helps to investigate the trophic structure inside nematode community. Different trophic groups of nematode demonstrated their varied ability to adapt to the environment in present study. Bacteriovores are considered as species insensitive or resistant to various disturbances of environment (Nagy *et al*., 2004). Cd-Hg 5+20 which could produce greater side effects presented highest proportion of bacteriovores (Table 4). Among all the bacteriovores, *Acrobeloides*, *Rhabditis* were the dominant genera, which are partially comparable with results by Zhang *et al*. (2011). Martinez *et al.* (2012) demonstrated that the series of Cd concentrations did not significantly affect *Acrobeloides*. In contrast, the abundance of *Acrobeloides* in contaminated soil was extremely lower than in unpolluted soil. The steep decrease of *Acrobeloides* may result from the fact that heavy metal which likely concentrated around rhizosphere lead to the irregular ultrastructural changes in esophageal and intestinal cells of nematode, which cause the conflict of nutrient absorption and digestion (Harada, 2006). Consequently, Cd can decrease body growth (Álvarez *et al*., 2006) and reproductive capacity (Harada, 2006), which contributed to the lowest values for both absolute and relative abundance of bacteriovores.

Plant parasites may be affected more by vegetation than contents of heavy metals (Šalamún *et al*., 2011). Plant parasites were the second abundant trophic group (Table 4), and their proportion peaked at Cd-5. Jin and Wang (2019) found Cd (<30 mg/kg) promoted the growth of plants, moreover Cd could combine with carboxyl functional groups, cellulose, proteins, lignin or hemicellulose on the cell wall to form precipitation, which helps plant parasites easily pierce the skin of roots and enhances their growth and reproduction. *Helicotylenchus* feeds mainly on the outside of the plant root (Sasser, 1989) only appeared at Cd-5 and Cd+Hg 5+20, our result was partially in accordance with findings by Šalamún *et al.* (2011) who reported *Helicotylenchus* was dominate genus in heavy metal polluted soil. Plant feeding nematodes *Paratylenchus* was negatively correlated with the heavy metals (Zhang *et al.*, 2007). Our data showed that *Paratylenchus* was the second abundant genus among all genera, its sub-adult stage made it have more advantage in adaptability. Ekschmitt *et al*. (2006) suggested *Paratylenchus* was a good candidate for substance-specific bioindication of Cr.

High ratio of fungivores compared with bacterivores may be a mark of heavy metal contamination (Bongers & Bongers, 1998). The highest proportion of fungivores at Hg-20 resulted in the highest value of f/b. Our finding was partially in line with Pen-Mouratov *et al*. (2008) who reported that fungivores and plant parasites were the most two abundant groups near the source of heavy metal pollution. Since the functional diversity and genetic structure of microbial communities could be influenced by dramatic increase of Hg (20 mg/kg) (Harris-Hellal *et al*., 2008), and fungi are more tolerant than bacteria to heavy metal pollution (Rieder *et al*., 2013), the relationship between fungivores and their food source fungi in polluted soil should be further investigated .

C-p group structure

Investigation of the c-p group structure of the nematode assemblage is a useful method to detect heavy metal pollution (de Goede *et al*., 1993; Bongers & Ferris, 1999). It is worthy to notice that bacteriovores with c-p1 nematodes were fewest at Cd-5, lower pH might result in the lack of c-p1 nematodes. Similar result can be found in the study of Sánchez-moreno and Navas (2007) who obtained that BF1 and BF2 nematodes were more abundant in the control than the polluted area. Omnivores-predators are always classified as high c-p value groups, as k-strategists they are sensitive to environmental changes, so omnivores-predator was the least abundance trophic group in this investigation. However, the proportion of c-p4-5 didn't show any regular change, this can be explained by the significant decline of the total abundance of nematodes in polluted soil. Šalamún *et al*. (2011) also discussed that the proportion of omnivores-predator was surprisingly relatively constant, fluctuating around 15 % at all sites. It is well known that c-p 3 group is more sensitive than c-p 1 and 2 group. Interestingly, nematodes classified as c-p 3 from different feeding groups all showed a tolerant response to disturbance rather than c-p 1-2 group at Cd-5. This finding is partially in accordance with Korthals *et al*. (1996) who reported that some c-p groups with lower values were as sensitive as groups with higher values. Another interesting phenomenon was that even the two genera have the same feeding type and c-p value, could have distinct responses to heavy metal pollution such as *Acrobeles* and *Acrobeloides*. Martinez *et al*. (2012) suggested that different bacterivorous species have different pollutant tolerances which influence their ecological interactions, then lead to a higher population fitness of one species under intensive pollution. The above analyses indicated that more deep investigations should be conducted to evaluate the relationship between the c-p groups and the sensitivities to disturbance especially in a short-term experiment.

Conclusions

After 3 months application, morning glories absorbed small part of heavy metals. Heavy metals had a deleterious effect on soil nematode assemblage, decreased nematode abundance, changed the structure of feeding groups and c-p groups. Single Hg seemed to have smaller impacts than Cd, and single Cd and Hg had fewer side effects than Cd-Hg on nematode communities. Direct analysis of nematode abundance, diversity, trophic and c-p groups could be more useful tools than some indices to assess the degree of soil disturbance in the short-term high level pollution experiment.

Conflict of interest statement

The authors declared that they have no conflicts of interest to this work. We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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