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Effect of Zinc Addition to Soil on Nematode Community Structure

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Zinc is essential for all life, and soil is the primary source of Zn in plant, animal and human nutrition. Enrichment of zinc in soil could cause damage to plant, animal and human through the food chains of ecosystem. City pollution (such as sewage, waste material and gas pollution) and land use (such as high inputs of fertilizers and agrochemicals) are the main reasons for zinc enrichments (Jiang *et al*. 2005).

To understand and evaluate the effects of heavy metal pollution on soil ecosystem, suitable indicators for assessing soil quality is necessary. Nematodes as bioindicators for soil health assessment had many merits (Bongers and Ferris 1999; Chen *et al*. 2003). Some studies indicated that nematode assemblage of agroecosystems was affected by metal positively and other studies proved negatively (Georgieva *et al*. 2002; Li *et al*. 2006). Li *et al*. (2006) studied the relationship between the soil nematode communities and soil Zn pollution in the field scale. Further study in controllable condition was conducted on the basis of the previous study. The objectives of this study were to evaluate the effect of Zn contaminated soil on the abundance, structure composition, diversity of nematode assemblage in a pot experiment, and to determine the relationship between soil nematode communities and soil Zn.

MATERIALS AND METHODS

The pot culture experiment was conducted in a greenhouse. Soil was collected from the top 10 cm of an arable field located in Shenyang Experimental Station of Ecology, Chinese Academy of Sciences. The soil collected is classified as meadow burozem (Chen *et al*. 2003), with 15.2 mg/kg total C, 1.36 mg/kg total N, pH 6.46. Fresh soil was passed through a 10 mm sieve to remove stones, stubble and coarse roots and then was allowed to dry for 2 days to decrease its water content to about 10%. Zinc in form of $ZnSO_4$ (purity 98%, Tianjin, China) was applied at rates of 0, 100, 200, 400, 800 mg/kg dry weight (they are denoted for CK, Zn 100, Zn 200, Zn 400, Zn 800). $ZnSO_4$ solutions were added to the pot soil and mixed with soil thoroughly. Plastic pots (20 cm diameter and 14 cm high) were filled with treated soils (2.5 kg/pot). Treated soil was kept for a period of 2 weeks, after which equilibrium between the added Zn and the soil was assumed. Twelve wheat seeds

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per pot were sown. Seedlings were thinned to 8 plants per pot after emergence. Soil samples were collected at the seedling (26 April), jointing (25 May), and ripening (6 July) stages of wheat growth in 2005. The experiment design was a complete randomized design with three replications.

The available Zn in soil was extracted by DTPA (diethylenetriamine penta-acetic acid, purity 99%, Shanghai, China) at pH 7.3. DTPA-extractable Zn was measured by atomic absorption spectrometry with a detection limit of 0.01 mg/L (Jiang *et al.* 2005).

Nematodes were extracted from 100 g soil sample (fresh weight) using the sugar flotation and centrifugation method (Li *et al.* 2006). All extracted nematodes in each sample were counted and expressed per 100 g dry weight soils according to the soil moisture (Liang *et al.* 2005). Nematodes in each sample were identified to genus level using light microscope. The classification of trophic groups was assigned to bacterivores, fungivores, plant-parasites and omnivore-predators based on known feeding habitats or stoma and esophageal morphology (Yeates *et al.* 1993).

Ecological indices of nematode communities were calculated: Shannon-Weaver diversity $H' = -\Sigma p_i \ln p_i$, evenness $J' = H'/\ln S$, dominance $\lambda = \Sigma p_i^2$, where p_i is the proportion of individuals in the ith taxon and S is the number of taxa (Yeates and Bongers 1999). Maturity index $MI = \Sigma v(i) \cdot f(i)$, where $v(i)$ is the *c-p* value of taxon i, $f(i)$ is the frequency of taxon i in a sample (Bongers 1990). Nematode channel ratio $NCR = B/(B+F)$, where B and F are the proportions of the nematode fauna allocated to bacterivorous and fungivorous groups. All data were subjected to statistical analysis of variance (ANOVA) in the SPSS statistical package.

RESULTS AND DISCUSSION

Across the wheat growth stages, DTPA-extractable Zn was significantly greater in the contaminated soils than in the control. DTPA-extractable Zn in soil increased with the application rate of Zn, and the percentage of Zn retained by soil ranged from 45% to 73% (Fig.1).

Effects of zinc on the numbers of total nematodes varied at different growth stages of wheat (Fig.2). There were no significant effects of zinc on the numbers of total nematodes at the seedling stage. At the jointing stage, the abundance of total nematodes was significantly lower in the Zn 400 and Zn 800 treatments than in the control. A significant reduction in the number of total nematodes only occurred in Zn 800 treatments at the ripening stage. During the study period, with the increasing concentration of Zn in soil, the effect of Zn on the abundance of total nematodes fluctuated gradually. Significant correlations were observed between the concentration of DTPA-extractable Zn in soil and the numbers of total nematodes across the jointing and ripening stages ($r = -0.65$, $P < 0.05$; $r = -0.74$, $P < 0.01$, respectively). In general, the numbers of total nematodes increased in the Zn 100 and Zn 200 treatments, but decreased in the Zn 400 and

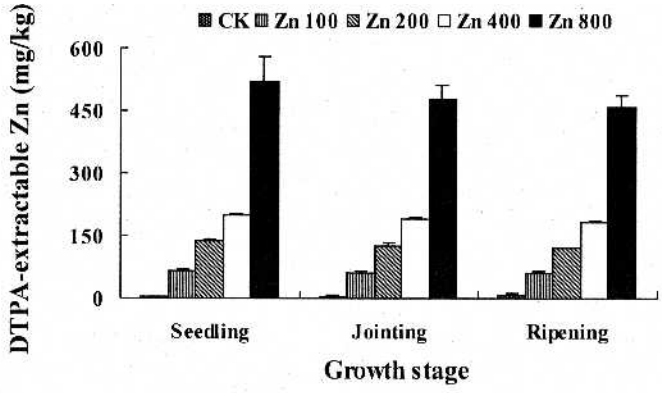

Figure 1. DTPA-extractable Zn in soil with different application rates of Zn

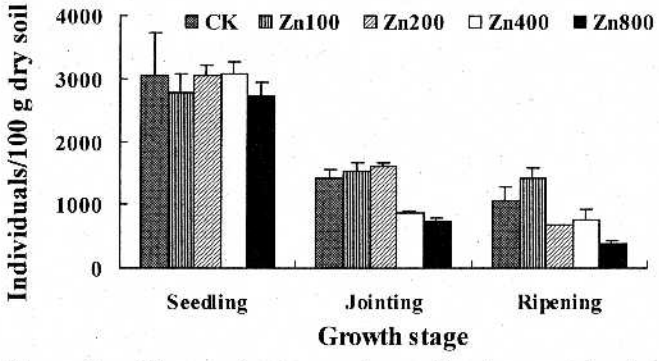

Figure 2. Changes in the numbers of total nematodes in soil with different application rates of Zn

Zn 800 treatments, as compared with the control. These results demonstrated that lower Zn concentration showed stimulative effect while higher concentration had inhibitive effect on the numbers of total nematodes. The observations were similar to that reported by Korthals (1998).

The numbers of bacterivores were significantly decreased at the seedling and jointing stages in the Zn 800 treatment compared to the control ($P < 0.05$) (Fig.3). Significant correlations were observed between the concentration of DTPA-extractable Zn in soil and the numbers of bacterivores at the seedling, jointing, and ripening stages ($r = -0.71$, $P < 0.01$; $r = -0.65$, $P < 0.05$; and $r = -0.61$, $P < 0.05$, respectively). These results also showed that bacterivore was the trophic group sensitive to different soil Zn levels (Li *et al.* 2005). Except for the ripening stage, there were no significant effects of DTPA-extractable Zn in soil on the number of fungivores. At the ripening stage, the number of fungivores increased significantly after exposed to Zn 100 treatments, but decreased at Zn 200, Zn 400, and Zn 800 treatments, as compared with the control. Yeates *et al.* (2003) found

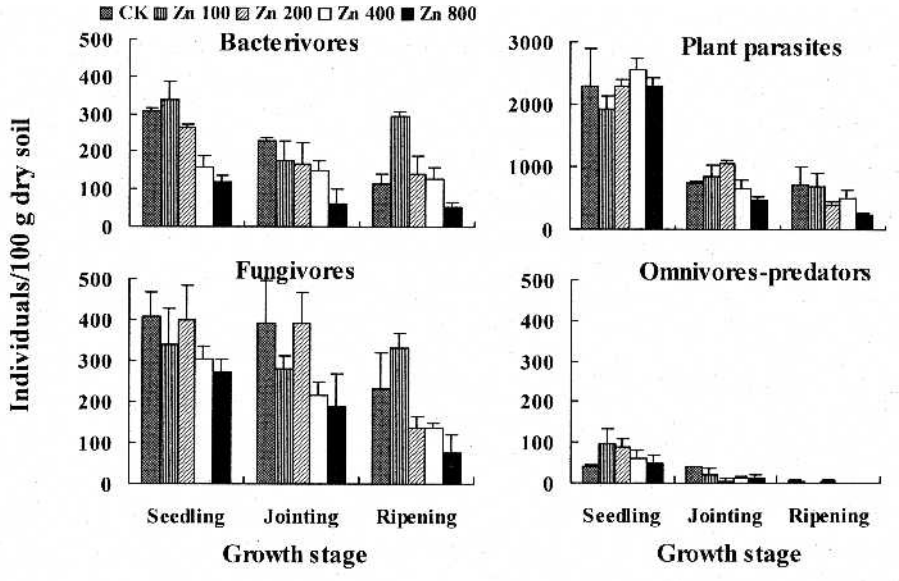

Figure 3. Distribution of nematode trophic groups in soil with different application rates of Zn

that zinc addition could significantly contribute to the variance in fungal-feeding nematodes. However, our results showed that the significant contribution appeared only in exposing low concentration pollutants during a certain period.

The number of plant parasites had no significant differences with different application rates of Zn, indicating that the abundance of plant parasites was not significantly affected by Zn addition in the whole growth period of wheat. However, Korthals (1998) reported that increasing Zn additions had negative effects on the proportion of plant feeding nematodes after one year. The omnivore-predator was the least tropic group in this study. A sharp decrease in the number of omnivores-predators was observed at the jointing and ripening stages. These results are in agree with Korthals (1996), who found that omnivores and predatory nematodes, known to be *K*- strategists, appeared very sensitive when exposed to certain concentrations of Zn.

Variation of ecological indices of soil nematodes in different treatments during the wheat growth period was shown in Table 1. Significant differences were observed in H' and λ during the wheat growth stages. J' was the least sensitive parameter for Zn contamination. *MI* was significantly affected by Zn addition only at the ripening stage. *MI* is usually used to indicate the stability and the extent being disturbed of ecosystem (Bongers 1990). These results demonstrated that the stability of ecosystem was not affected in early period of wheat growth and the effect of pollutants only occurred after a certain period of time. Significant correlations were observed between the concentration of DTPA-extractable Zn in

Table 1. Variation of ecological indices of soil nematodes in contaminated soil with different application rates of Zn (means ± standard error)

Treatment		*H'*	*λ*	*J'*	*MI*	*NCR*
Seedling (26 April)	CK	1.61±0.07	0.30±0.03	0.61±0.03	2.07±0.06	0.41±0.04
	Zn100	1.83±0.12	0.26±0.03	0.67±0.03	2.20±0.07	0.34±0.06
	Zn200	1.51±0.05	0.36±0.05	0.60±0.03	2.18±0.02	0.30±0.04
	Zn400	1.26±0.01	0.44±0.02	0.54±0.02	2.20±0.08	0.39±0.05
	Zn800	1.29±0.03	0.41±0.01	0.53±0.01	2.23±0.02	0.56±0.05
	P value	<0.01	<0.05	<0.05	ns	<0.05
Jointing (25 May)	CK	1.83±0.03	0.24±0.00	0.65±0.01	2.07±0.06	0.30±0.09
	Zn100	1.53±0.07	0.32±0.01	0.64±0.01	2.20±0.07	0.41±0.04
	Zn200	1.41±0.09	0.34±0.04	0.65±0.04	2.18±0.02	0.23±0.10
	Zn400	1.59±0.10	0.28±0.03	0.65±0.02	2.20±0.08	0.41±0.05
	Zn800	1.40±0.08	0.35±0.03	0.64±0.04	2.23±0.02	0.38±0.10
	P value	<0.05	<0.05	ns	ns	ns
Ripening (6 July)	CK	1.40±0.03	0.34±0.02	0.64±0.02	2.00±0.02	0.41±0.11
	Zn100	1.71±0.08	0.24±0.02	0.73±0.01	1.99±0.01	0.41±0.01
	Zn200	1.76±0.09	0.22±0.04	0.76±0.05	2.05±0.03	0.57±0.08
	Zn400	1.59±0.16	0.27±0.06	0.73±0.04	1.99±0.01	0.47±0.08
	Zn800	1.63±0.10	0.24±0.04	0.73±0.03	1.88±0.01	0.48±0.08
	P value	<0.01	<0.05	ns	<0.01	ns

ns, not significant.

soil and the values of *H'* in wheat growth period. At the seedling stage, values for *NCR* were significantly correlated with the concentrations of DTPA-extractable Zn in soil (r = -0.543, $P < 0.05$). Our results showed that the link between soil Zn and nematode assemblage really existed and this link could be used to reflect the indirect effects of soil Zn on ecosystem-level processes.

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