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Effects of Heavy Metals and Organic Matter on Nematode Population in Soil
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With best regards,
Prof. Giancarlo Renella, Conference chair of the 11th ICOBTE

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Effects of heavy metals and organic matter on nematode population in soil

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Abstract
Heavy metals are categorized as one of the most important contaminants that disturb soil environment and thus are harmful for terrestrial organisms. Nematodes as an indicator organism may be a suitable index to assess heavy metals risks in the soil. Also, organic compounds such as manure have an important role in nematode population traits. The objective of this study was to investigate the impact of different levels of heavy metals (Cd, Ni, and Pb) and organic matter on nematode population characteristics of soil. Therefore, a completely randomized factorial design was carried out including two levels of organic matter 0 (O0) and 1% (O1) and three levels of heavy metals 0 (H0); 100, 500, 1000 (H1); and 300, 700, 2000 (H2) mg kg$^{-1}$ of Cd, Ni, and Pb, respectively. All treatments were incubated for three months at 27°C and 70% of field capacity soil moisture. After 30 and 90 days incubation time, the total population, juvenile and mature numbers of nematodes were determined. The results showed that total population, juvenile and mature numbers of nematodes significantly decreased by addition of heavy metals. The results also revealed that the adverse effects of the metals may be masked by organic matter application.

Introduction
Nowadays development of industries, advanced anthropogenic activities and use of various pollutants lead to disturb terrestrial ecosystem. Heavy metals are one of the prevalent pollutants threatening soil health in the environment. Furthermore, application of organic matter is a common practice for the reclamation of soils. Monitoring soil quality by means of biological characteristics, especially by investigating contaminants effects on soil organisms has been considered in recent studies. Since soil nematode communities are the most abundant metazoan that play an important role in food chain (Sochova et al., 2006); they can be recommended as an effective tool to examine adverse impacts of heavy metals in soil. The objective of this research was to study of effects of heavy metals (Ni, Cd, Pb) and organic matter (manure) on abundance and community structure of nematodes in soil under controlled condition.

Materials and Methods
Soil samples were collected from Golestan province located in the north east of Iran. The experiment was carried out in a completely randomized factorial design with three replicates. Soil was polluted with mixture of heavy metals (HMs) as Cd(NO3)2·4H2O, Pb(NO3)2 and NiSO4. Thus, total elements concentrations added to soil were 0 (H0); 100, 500, 1000 (H1); and 300, 700, 2000 (H2) mg kg$^{-1}$ of Cd, Ni, and Pb, respectively. Two levels of organic matter 0 (O0) and 1% (O1) was applied to the soil. The soil samples were moistened to and kept at 70% of field capacity during the incubation periods (30 and 90 days). For nematological analysis, they were extracted from the soil by wet sieving followed by centrifugation (Jenkins, 1964). Then extracted nematodes were killed by heat and fixed on slides using procedures developed by Sinhorst (1959) and De Grisse (1969). Nematodes were counted in all treatments by applying Korthals and Bongers key under light microscopy.

Results
The results showed that total nematode number in control treatment (H0O0) was 400 per 200 g of soil samples. When organic matter was applied to soil samples, the nematode number increased to 800 per 200 g of soil samples. In low and high level of heavy metals treatments (H1 and H2) nematode number significantly decreased after three months of soil samples incubation. Nematodes number in H2 treatments were 150 and 400 per 200 g for low and high level of organic matter treatments, respectively. Reduction in nematodes number was not as much of those treatments when organic matter was applied to soil samples (Figure1). Juvenile and mature nematodes number showed the same trend as mentioned for total nematode number (Figure 2 and 3). In O1 treatments (1% organic matter) the juvenile and mature
nematode numbers were higher compared to those without organic matter (O). The results also showed that the positive effects of organic matter on nematode population were reduced by increasing heavy metals concentration in soil samples. It seems that toxicity of heavy metals on nematode population was covered by organic matter. The results also demonstrated that juvenile number was affected more than mature nematode number in treatments with high level of heavy metals (H).

**Figure 1.** Changes of total population of nematodes in soil samples treated with heavy metals

**Figure 2.** Changes of larve (Juvenile) population of nematodes in soil samples treated with heavy metals

**Figure 3.** Changes of mature population of nematodes in soil samples treated with heavy metals

**Discussion**

Our results suggested that heavy metals were toxic to nematodes. Total population and juvenile and mature nematodes number were reduced significantly in the presence of high concentration of heavy metals. Similar observation was reported by Pen-Mouratov et al. (2008). It seems that mature nematodes are more sensitive to heavy metals compared to juvenile nematodes. Organic matter controlled the toxicity of heavy metals and detrimental effect of heavy metals was masked with application of organic matter. As nematode population characteristics responded to heavy metal concentration, it seems that it can be a useful tool to assess soil ecosystem disturbance.

**References**


Seinhorst, J.W. 1959. A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. Nematologica, 4:67-69