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Determination of Lead Accumulation in the *Lumbricus terrestris* (earthworm) living in the Roadside Soils of Eskişehir

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Abstract

The study was conducted on *Lumbricus terrestris* (earthworm) in order to determine the level of vehicle derived lead contamination on living. *Lumbricus terrestris* specimens were analysed for their lead content by using Hitachi (180-70) Polarized Atomic Absorption Spectrophotometer. Lead salts were searched in tissues according to the Rhodizonate Method for Lead Salts. Lead concentrations of the specimens taken from traffic dense areas were higher than the specimens taken from parks.

Key words: *Roadside, soil, earthworm, lead*

Özet

Bu çalışma, toprak solucanlarında Lumbricus terrestris trafikten kaynaklanan kurşun birikmesi düzeyini belirlemek amacıyla yapılmıştır. Lumbricus terrestris örneklerindeki kurşun miktarı Hitachi (180-70) Polarize Atomik Absorbsiyon Spektrofotometresi kullanılarak incelenmiştir. Dokulardaki kurşun birikimi Rhodizonate Yöntemi ile araştırılmıştır. Trafiğin yoğun olduğu bölgelerden alınan örneklerdeki kurşun miktarı parklardan alınan örneklerdekinden daha yüksek çıkmıştır.

***Anahtar Kelimeler**: Yol kenarı Toprak, Toprak Solucanı, Kurşun*

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1. INTRODUCTION

Lumbricus terrestris probably takes three to four years to mature but this slow-growing species is long-lived, with adults surviving in cultures for up to thirty years. The natural longevity of most earthworms is short with small species unlikely to survive the hazards in natural habitats for for more than 12 years (Sims and Gerard, 1999).

Lead in soil results from various municipal and industrial wastes, automobile emissions, decomposition of paints in aged homes and sewage waste (Grandjean, 1992). It has been reported that human, animals and plants living close to main roads with heavy traffic were affected by lead and that their tissues contain more of this heavy metal than the tissues of those who live remote from main roads (Manay, 1999; Swaileh, 2001). At the first part of the study we found elevated levels of lead in the roadside soils and plants (Işıklı, 1999).

Earthworms have great potential for use as bioassay/biomonitor organisms in studies of contaminant uptake and possess many characteristics that make them ideally suited for this purpose (Ma, 1982). Studies have demonstrated that native species of earthworms, collected at contaminated sites, can be used to indicate biologically available levels of these contaminants (US Army Engineer Waterways Experiment Station, 1988).

Ireland reported that earthworms could accumulate in their tissues heavy metals in contaminated environment (Ireland,1983). That earthworms could serve as bio-indicators for metal pollution was shown by Morgan and Morgan (Morgan, 1993) and Stafford and McGrath (Stafford, 1986) who reported significant positive correlation between the earthworms and total soil lead concentration from various metal contaminated sites.

At the second part of the study, we aimed to determine the level of vehicle derivate lead contamination on the living.

2. MATERIALS AND METHODS

The second part of the study was conducted on *Lumbricus terrestris* (earthworm) in order to determine the level of vehicle derived lead contamination on the living.

The *Lumbricus terrestris* samples were taken from the same places as the soil and plant samples taken.

The earthworms, identified as *Lumbricus terrestris* were collected by spraying the soil with 0.05% formalin and washed free of adhering soil particles and placed in petridishes then refrigerated at 10°C for 24 hours in order to purge the soil in the gut, thereafter were then removed and rinsed slightly with deionized water and then frozen pending analysis as described by Bamgbose et al (Bamgbose, 2000).

Each of the sample groups were divided into two groups.

The samples in the first group: 3 g of the thawed earthworm samples were weighed and digested with 2ml concentrated nitric acid and heated to dryness on a hotplate. The digest was redissolved in 1 ml concentrated nitric acid after which it was made up to 50 ml with distilled water as described by Bamgbose et al. The sample digests were analyzed for lead contents by using Hitachi (180-70) Polarized Zeeman Atomic Absorption Spectrometer. All the samples were analyzed in triplicate and the mean values were calculated. Results were used in terms of avarage values ± standart deviation (x±sd). Statistical analyses were based on "t tests for independent samples".

Histological sections were taken from the second set of earthwarms and lead salts were searched in tissues according to the "Chromate Method for Lead Salts" (Everson, 1961) and "Rhodizanate Method (Bancroft, 1977) for Lead Salts" at the beginning of the study. Both of the methods were successful for showing lead salts in tissues, so we continued by the "Rhodizanate Method for Lead Salts".

3. RESULTS AND DISCUSSION

For the subject areas, the average lead concentrations of the non-washed and washed surface (0-15 cm depth) soil samples were 46.69 ± 14.70 mg/g and 45.36 ± 14.58 mg/g, respectively, and for the deeper (15-30 cm depth) layers, they were 46.01 ± 12.54 mg/g and 41.03 ± 11.49 mg/g, respectively. The observed concentrations were decreasing with washing and depth. It was similar to the results of Manay et al (Manay, 1999) and Burguera et al (Burguera, 1988).

For control areas the average lead concentrations of non-washed and washed surface soil samples were 11.51 ± 1.11 mg/g g and 10.49 ± 1.48 mg/g, respectively, and were 16.28 ± 15.69 mg/g and 8.90 ± 1.16 mg/g, respectively, for the deeper layers.

The average lead concentration of surface soil samples from roadsides with heavy traffic *(46.69±14.70* mg/g*)* was significantly higher than that of control areas *($t=5.24$, $p<0.05$)*. The average lead concentration in control areas *(11.51±1.11* mg/g*)* was close to 12 ppm that is given by Grandjean (Grandjean, 1992) for earth's crust.

The average lead concentration of grass type plant samples, taken from the same places where soil samples were collected, was 15.49±8.35 mg/kg for subject samples and 2.69±0.96 mg/g for control samples. A significant difference was observed between the subject and control groups *($t=3.35$, $p<0.05$)*. Significant differences were observed, regarding washing, in both the subject and control plants *(respectively, $t=7.29$, $p<0.05$; $t=4.51$, $p<0.05$)*.

Significant differences in the direction of decrease were observed in soil and plant specimens taken from the subject and control areas regarding the data obtained on wet season when there was heavy rainfall and dry season when the soil was dry. Although the fact that the lead concentration decreases in both the soil and plant samples by washing while a higher lead concentration was present on rainy days than when the soil was dry, may seem contradictory, this situation may most possibly results from the dusts on the roads with a high lead concentration being carried by rain to roadside soils. Othman et al reported that lead levels were lower in soil samples during the wet period (December to April) whereas, it was higher in plants during the same period (Othman, 1997).

General populations living near main roads or in areas of heavy traffic are in risk groups. We wanted to evaluate this finding from the point of view of living. But it is impossible to determine this point in human because of the difficulties on calculating the exposure level via the roadsides. Also it is impossible to determine this in animals because of the same reason. So, for this reason we used earthworms to demonstrate the lead pollution as announced by Morgan and Morgan (Morgan, 1993) and Davies NA et al (Davies, 2001) that earthworms could serve as bio-indicators for metal pollution.

The average lead concentration of the earthworms taken from the roadside soils was 40.16 ± 17.57mg/g with a range of 21.14mg/g and 67.03mg/g. The mean lead concentrations of the earthworms were lower than those of the mean soil samples. This conforms to the work of Bamgbose who reported lower levels of lead in earthworms from polluted areas. The lead concentrations of the earthworms were irrespective of the lead concentration of the soils. The percentage of lead concentration in earthworm samples to soil samples was computed based on the mean concentrations of lead in the subject areas and were found to range between 74% to 88%. The percentages were similar to those obtained by Bamgbose et al and Saciragac et al (Saciragac, 1990).

For control sites the average lead concentration of the earthworms was 12.35 ± 1.61 mg/g with a range of 10.22 mg/g and 14.03mg/g. In contrast to subject sites the mean lead concentrations of the earthworms were slightly higher than those of the mean soil samples. This was harmonious with the findings of Bamgbose et al. Significant difference was found between the lead concentrations of worms taken from the subject and control sites (t=3.467, p=0.004).

Areas of studying	Pb analysis
1	21,14
2	24,36
3	53,55
4	28,68
5	42,36
6	60,28
7	24,65
8	55,32
9	24,23
10	67,03

Areas of control	Pb analysis
1	10,22
2	11,26
3	12,54
4	13,68
5	14,03

In the histological sections, taken from the second set of earthworms, lead salts were demonstrated clearly and the one containing 10.22 mg/g lead is given in Figure 1 and the one containing 67.03mg/g lead is given in Figure 2.

Lead concentrations of the specimens taken from traffic dense areas were higher than the specimens taken from parks.

It is also concluded that the earthworm can serve as a bio-indicator in the environment.

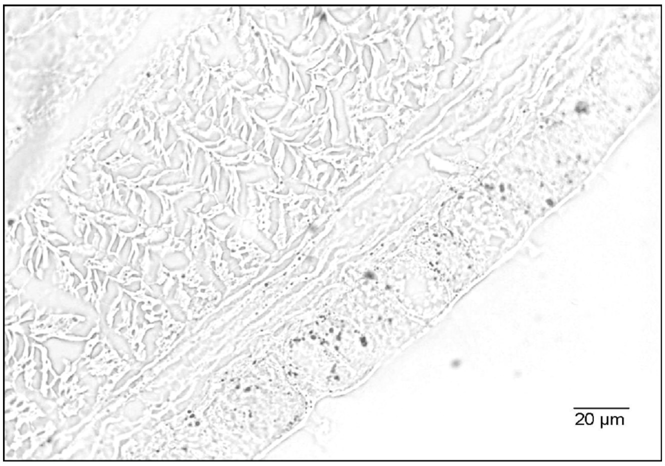

Figure 1: *The histological sections, taken from the earthworms from control areas, the one containing 10.22 mg/g lead*

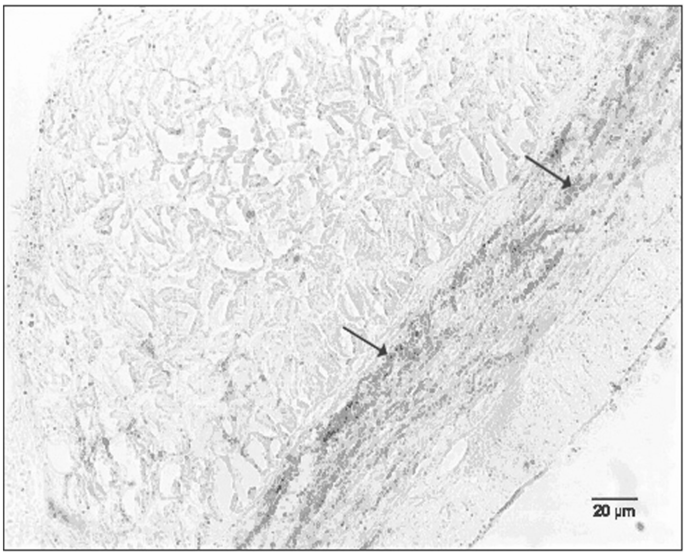

Figure 2: *The histological sections, taken from the earthworms from subject areas, the one containing 67. 03µg/g lead*

The authors recommend more systemic clinical examination of the population living in risk areas to determine the potential health risks and to officially recognize lead pollution as an environmental problem as mentioned by Pagotto C et al (Pagotto, 2001).

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