

## EFFECTS OF ENVIRONMENTAL POLLUTANTS on *Lumbricus terrestris* 7-ETHOXYRESORUFIN-*O*-DEETHYLASE (EROD) ENZYME ACTIVITY

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### ABSTRACT

Polluted water, atmospheric poisonous gasses, urban and industrial wastes passes over the cavities and affects the organisms living inside of the soil. Polycyclic aromatic hydrocarbons and polychlorinated biphenyls are two of the most important groups of these pollutants.

The effects of environmental pollutants on *Lumbricus terrestris* samples were determined by the fluorescent spectrophotometric measurement of 7-Ethoxyresorufin-*O*-deethylase (EROD) activities catalysed by CYP4501A1. Along the sampling sites between Eskişehir and Kütahya, the results suggested the induction of the enzyme activity according to the intensity of agricultural activities, industrialization and urbanization levels of the regions. The results clearly show the influence of industrial complexes near Kütahya and Çukurhisar.

**Key Words:** EROD, Earthworm, Environmental Pollution, Fluorescent Spectrophotometry

### *Lumbricus terrestris* 7-ETOKSİREZORUFİN-*O*-DEETİLİZ (EROD) ENZİMİ AKTİVİTESİ ÜZERİNE ÇEVRESEL KİRLETİCİLERİN ETKİLERİ

### ÖZET

Kirli sular, atmosferdeki zehirli gazlar, kentsel ve endüstriyel atıklar topraktaki boşluklardan sızarak, toprak içerisindeki organizmaları etkilemektedir. Polisiklik aromatik hidrokarbonlar ve poliklorobifenilli bileşikler bu kirleticilerin en önemli gurubunu oluşturmaktadır.

Çevresel kirleticilerin *Lumbricus terrestris* üzerindeki etkileri, CYP4501A1 tarafından katalizlenen Etoksirezorufin-*O*-deetilaz (EROD) enzim aktivitesinin florasan spektrofotometre ile ölçümü sonucunda belirlenmiştir.

Elde edilen sonuçlar, Kütahya ve Eskişehir illeri arasındaki örnekleme alanı boyunca, tarımsal aktivite, endüstrileşme ve kentselleşme düzeyine bağlı olarak enzim aktivitesinin indüklendiğini göstermektedir. Sonuçlar, Kütahya ve Çukurhisar yakınlarındaki endüstriyel bölgelerin etkisini açıkça ortaya koymaktadır.

**Anahtar Kelimeler:** EROD, Toprak solucanı, Çevresel kirlilik, Floresan Spektrofotometre

## 1. INTRODUCTION

Although pollution is usually considered as an anthropogenic stress factor, many other organisms including aquatic species and earthworms are subjected to several harmful effects. Polluted water, poisonous gasses in the air and urban wastes pass over the cavities and affect the organisms inside the soil. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) are the most important groups of these pollutants. PCBs are industrially sourced ubiquitous contaminants of the environment. Their manufacture included routine releases into the environment. PAHs are formed by combustion and pyrolysis of fossil fuels and other organic materials. PAHs and PCBs are found to be activated to reactive intermediates that ultimately result toxicity, carcinogenicity and mutagenicity on their oxidation steps [1, 2, 3].

A part of these harmful substances are excreted immediately over the gill, kidney or skin. Another part will be detoxified through a metabolizing system in the liver. Cytochrome P-450 mediated mixed function oxidases (MFO) or monooxygenases (MO) which constitute one family of the phase I enzymes are such a metabolizing system located in the endoplasmic reticulum for protecting cells from lipophilic xenobiotics. They convert harmful substances into more polar metabolites which are easily excreted and are involved in the metabolism of endogenous compounds such as steroids, bile acids, fatty acids and of innumerable exogenous compounds including drugs, carcinogens and environmental chemicals [4, 5].

Monooxygenases all belong to a gene superfamily, all acting on various substrates. One of them can be measured by using 7-ethoxyresorufin as an artificial substrate. 7 Ethoxyresorufin-O-deethylase (EROD) activity has been used as a biomarker in detecting of fish and wildlife to environmental contaminants. This reaction is catalyzed by cytochrome-P4501A1 and indicates the induction of cytochrome P4501A1 detoxifying enzymes which is measured by various methods [6, 7, 8, 9].

In this study, EROD activity of the earthworms, *Lumbricus terrestris*, is assayed. Samples were collected from 6 stations inside the region from Eskişehir to Kütahya in Turkey. The results are compared by using student-t test of SPSS statistical program.

## 2. MATERIALS AND METHODS

### 2.1. Animal

*Lumbricus terrestris* were sampled on November 2002 from the 6 sampling sites from Eskişehir to Kütahya. They were kept in the soil which was taken from the stations that they naturally exist. Appropriate moisture and temprature conditions were regulated at least 7 days to accustom the animals to the laboratory conditions and then immediately used in the experiments.

### 2.2. Sampling Sites

The sampling sites between Eskişehir and Kütahya are given in Figure 1. These sites were chosen according to agricultural activities, different urbanization and industrialization levels that may cause variations in the enzyme activity. The station Kızılınler, that is possibly the least effected from the pollutants, was chosen as the control group.

There is a cleaning facility on the Porsuk River between station A and station B which are out of Eskişehir. Pollution status of the Porsuk River was considered for the soil contaminated with the river water (A: Before the cleaning facility, B:After the cleaning facility).

CONTROL:Kızılınler, A:Before the cleaning facility, B:After the cleaning facility, C:Yunus Kent/Eskişehir, D:Kütahya, E:Çukurhisar localities are between Eskişehir and Kütahya.

### 2.3. EROD Activity

For enzymatic studies, each animal (17 samples for each station) was homogenized on ice with a teflon pestle and Potter-Elvehjem glass homogenizer after addition of 0.1 M Tris-chloride buffer, pH, 7.8. The homogenate was centrifuged 20.000 rpm for 5 min at 0 °C in Heraeus-Spatech, Varifuge 20RS centrifuge. The pellet was discarded and the supernatant was used for measuring EROD activity. EROD activity was determined by spectrofluorometric method of Prough, Burke and Mayer, 1976 [6] with some modifications. Protein concentrations were determined

by using the method described by Lowry et al., [10]. Experiments were carried out duplicate.

The incubation mixture for ethoxyresorufin dealkylation reaction consists 0.1 ml of microsomal protein (0.1-1.0 mg/ml) and 1.8 ml of 0.1 M Tris-chloride buffer, pH 7.8. After adjusting the excitation and emission wavelengths to 530 and 585 nm, respectively, the rate of fluorescence change vs time is recorded prior to adding 10 µl of NADPH in a final volume of 2.0 ml. The reaction was initiated by the addition of substrate and followed for 2 min in a Shimadzu (RF 5301 PC) spectrophotofluorometer.

#### 2.4. Statistical Analysis

EROD activity of *Lumbricus terrestris* from control group and from other stations were compared by using student-t test.

### 3. RESULTS

*Lumbricus terrestris* samples from the 6 sampling sites that are thought to have different pollution status according to their agricultural activities, different urbanization and industrialization levels were analyzed for the EROD activity. The station Kızılınlar, that is possibly the least effected from the pollutants, was chosen as the control group.

Several industries are located in Eskişehir and Kütahya. The main source of pollution in Eskişehir includes untreated or partially treated domestic and industrial wastes, urban and agricultural run-off, sediments and contaminated waters from rivers.

There is a cleaning facility on the Porsuk River between station A and station B which are out of Eskişehir. Pollution status of the Porsuk River was considered for the soil contaminated with the river water (A: Before the cleaning facility, B:After the cleaning facility).

The comparison of control group with other 5 stations was performed by using student-t test of SPSS statistical program. The results are shown in Table 1.

An increase in the enzyme activity was observed for 4 of the stations when compared to the control group. There is a cleaning facility on the Porsuk River between the station A and station B which are out of Eskişehir. The enzyme activity of the earthworms collected from station A was significantly

higher than the earthworms from station B. Stations D and E are the potentially polluted regions because of the intensity of industrial complexes. The results supported this opinion with the induced enzyme activity of the earthworms. Station C is the region of urbanization and as expected, there was an induction in the enzyme activity of the earthworms from this site.

#### 4. DISCUSSION

Generally metabolic pathways and reactions allow organisms to persist under adverse environmental conditions and can be used as biomarkers to characterize either the exposure situation itself or effects induced by the stress in exposed organisms [11].

One rapid and sensitive method is based on an assay of mixed function oxidase (MFO) activity, i.e. the cytochrome P-450 dependent monooxygenase system involved in the metabolism and transformation of both endogenous and xenobiotic organic aromatic compounds. The MFO enzymatic system and particularly the ethoxyresorufin *-o*-deethylase (EROD) activity, can be induced by PAHs and PCBs. EROD assay is currently considered as a simple and reproducible tool for the assessment of the presence of hydrocarbons in the environment ranging from severe pollution down to low level contamination [12].

In the present study, EROD activity, that can be affected either from PAHs and PCBs, was investigated in the earthworm *Lumbricus terrestris*.

The results have shown that EROD activity was increased in stations A, C, D and E when compared to the control group. The Porsuk River covers a significant urban, agricultural and industrial area of the region, along its basin from Kütahya to Eskişehir. The soil before the cleaning facility was thought to be contaminated as a result of usage of the river water in agriculture. Induced EROD activity was measured in the earthworms from station A. Industrial complexes near Kütahya and Çukurhisar were the potential source of PAH contamination of the soil in stations D and E. These are the sites that contain the most effected organisms according to the enzyme induction. The enzyme induction in station C can also be explained as a result of PAHs from exhaust gasses of the cars and chimney fumes of the houses according to the urbanization.

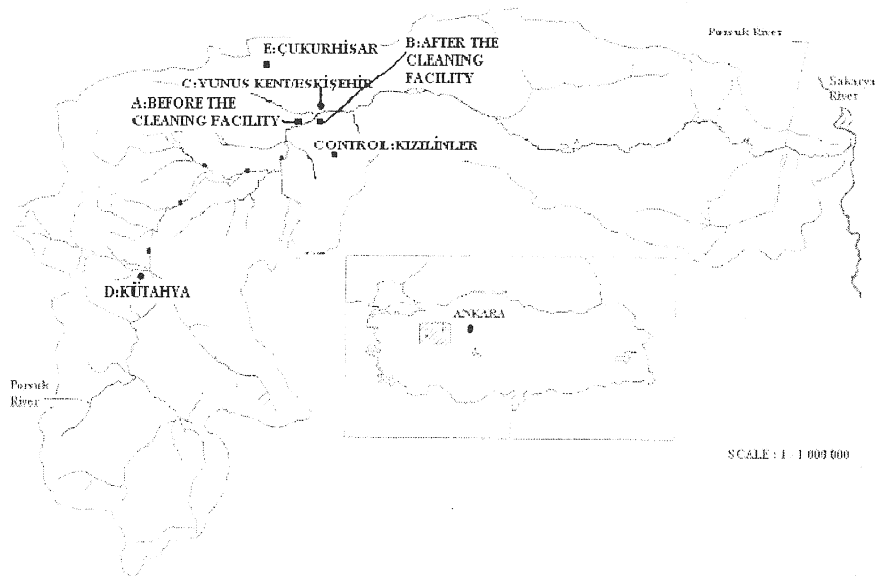
The present observations show the influence of the industrial complexes in Kütahya and Çukurhisar, urbanization and agricultural activities on

environmental pollution, by determining the accumulation of contaminants, on the base of the usage of EROD activity as a biomarker response.

In the further studies, investigations about PAH and PCB and heavy metal levels of the sampling sites, could be useful in order to correlate the enzyme induction with pollution levels.

**Table 1.** Comparison of EROD activity of *Lumbricus terrestris* from control group and from other stations. Data are the mean of at least two replicate analyses as nanomoles resorufin  $\text{min}^{-1}$ ,  $\text{mg protein}^{-1} \pm \text{SD}$  values  
\* $p \leq 0.05$  (student-t test)

Stations	EROD Activity
Control	0.012 $\pm$ 0.003
A	0.024 $\pm$ 0.008*
B	0.018 $\pm$ 0.014
C	0.017 $\pm$ 0.002*
D	0.033 $\pm$ 0.009*
E	0.035 $\pm$ 0.009*



**Figure 1.** Sampling sites in the study area.

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