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EROD and metallothionein in *Limnodrilus profundicola* (Oligochaeta: Tubificidae) as an indicator of pollution exposure in the Curuksu stream of Menderes river, Denizli–Turkey

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ABSTRACT

In this study, pollution in Curuksu Stream of Menderes River has been followed by measuring the chemical and biological parameters on five predetermined stations for about 20 mon. Our previous studies have shown that *Limnodrilus profundicola*, *Eristalis sp.* and *Chironomus thummi* taxa are valuable bioindicators for the Curuksu stream and *Limnodrilus profundicola* Metallo-Thionein (MT) and EthoxyResorufin-O-deEthylase (EROD) levels are valuable and useful biomarkers for biomonitoring heavy metals and PAHs pollutions, respectively. Cu-, Cr-, Cd- and Pb-type pollution was detected in all sediment samples taken from all of the stations, including reference station. Pollution source of these heavy metals are considered to be industrial wastewater, atmosphere and soil. Similarly, PAHs level was found to be considerably higher for Curuksu and Guzelkoy stations than other stations. These heavy metal- and PAHs-pollution were also confirmed with elevated MT and EROD levels measured with *Limnodrilus profundicola* sampled from corresponding stations. Industrial wastewaters, coal, exhaust gas and forest fires are among the expected sources of PAHs-type pollutants in Curuksu stream. This heavy pollution seen in Curuksu and Guzelkoy stations could probably have arisen from receiving more wastewater than other stations and there would not be enough time for natural purifications. In conclusion, our results clearly stated that food chain has completely broken for Curuksu and Guzelkoy stations which are also extensively polluted with heavy metals and PAHs.

Keywords: Bioindicator organism; Biomarker; Curuksu stream; Heavy metal pollution; PAHs pollution

1. Introduction

Aquatic ecosystems are constantly exposed to urban, agricultural or industrial pollutants which are mostly adsorbed by suspended particles and subsequently accumulated in the sediments [1,2]. Contaminants including polycyclic aromatic hydrocarbons (PAHs) polychlorobiphenyls (PCBs), organophosphates, organometals, thiocarbamates and metals are biologically available and uptake is proposed to occur from the sediment, suspended particulate matter, water-column and food sources [3–9]. Muddy sediments are known to accumulate hydrophobic contaminants such as PAHs to a much greater extent than sands [10,11]. In addition, sediments can accumulate metals at concentrations 10,000 times higher than in the overlying water column, constituting an important source of contamination and risk for living organisms. Metals are a well-known pollutants causing environmental degradation in rivers and coastal aquatic systems [12]. Particularly, Cu and Cd are two important xenobiotics in aquatic ecosystems as well as being

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non-degradable and cumulative pollutants, which exert a wide range of pathologic effects on fish and other aquatic organisms [13,14]. All aquatic invertebrates accumulate trace metals in their bodies whether or not these metals are essential to metabolism [15].

Chemical analyses of heavy metals or PAHs characterize the contamination level of the medium (water or sediment), but they are inadequate to assess the biological quality of a zone being studied. Only living organisms are able to integrate the various complex effects of contaminants that are really bioavailable [12,16]. Monitoring living organisms at different levels of biological organization is the main tool for investigating the health of an ecosystem. Especially, biomarkers that employ enzyme activity measurements which can detect low levels of pollution are heavily used in ecological risk assessments of aquatic ecosystems. These biomarkers have the potential to identify the incidence of exposure to, and effects caused by, contaminants and so they provide an early warning of potentially damaging effects at higher levels of biological organization [17].

Biomarker, CYP1A, and particularly EROD activity, which is more specifically induced by PAHs, PCBs and dioxins play a crucial role in the detoxification and metabolism of a variety of endogenous and xenobiotic compounds, including many environmental pollutants [18]. CYP1A induction is a sensitive and specific adaptive response of organisms exposed to environmental pollutants such as planar congeners of polychlorinated dibenzodioxins (PCDD), PCBs and several PAHs [19]. The inducibility and/or suppressibility of the isoenzyme CYP1A catalyzed EROD activity by such environmental pollutants as PAHs, PCBs and OP insecticide suppression is the basis for its usefulness in field monitoring [20–22]. Therefore, measurement of EROD activity are using as the exposure index, thus enabling the identification of areas contaminated by industrial or domestic pollutants [18].

MTs comprise a class of inducible metal-binding non-enzymatic proteins characterized by a low-molecular-mass thermo-resistant proteins with a high cysteine content (approximately 30%) and affinity with various metals and a wide distribution in various organisms including mammals, fishes and invertebrates [12,14,23–26]. MTs play a major role in the homeostasis of essential metals (such as Zn and Cu) and also in detoxification of non-essential metals such as Ag, Hg and Cd [12,24–29]. High metal concentration in cell induces an increase in MT concentration [25,27] and the use of MTs as a biomarkers of Ag, Cd, Cu, Hg contamination has been evaluated by several authors for different animal species such as annelids, molluscs, crustaceans, fish [24,25,27]. Therefore, MTs are widely considered as biochemical environmental indicators of metal contamination [26,30,31].

The aim of this study is to find out the PAHs and heavy metal pollution levels in Curuksu Stream by using a bioindicator organism *L. profundicola* and by measuring the chemical and biological parameters.

2. Materials and methods

2.1. Materials

In this study, water and sediment samples were collected from Saricay, Curuksu, Guzelkoy, Korucuk and Sigma stations on Menderes River which is located in the West of Turkey and has been polluted by industrial discharges and agricultural processes (Fig. 1). The freshwater oligochaete *L. profundicola* (Verril, 1871) was chosen as a test organism. It is endobenthic species living in the mud of estuaries and feed on sediment. Therefore, exhibits maximum contact with the substrate in/on the sediment.

2.2. Chemical analysis of water and sediment samples

Cu, Cr, Cd and Pb levels of water and sediment samples were determined by atomic absorption spectrophotometer (AAS). For this purpose, 1 g of dried sediment sample was treated with 10 ml of HCl:HNO_3 (3:1) mixture at 70 °C for 6 h. The suspension was filtered and diluted to 25 ml with distilled water for the analysis [32].

2.3. Polycyclic aromatic hydrocarbon (PAH) analysis

PAH analyses of water samples were performed by Shimadzu LC–20 AD Prominence High Performance Liquid Chromatography System (HPLC) (Shimadzu SPD-M20A

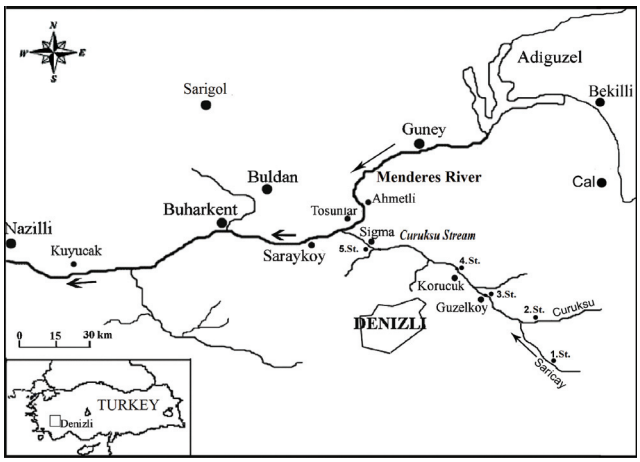

Fig. 1. *L. profundicola* sampling stations on Menderes River. St.1: Sampling station Saricay; St.2: Sampling station Curuksu; St.3: Sampling station Guzelkoy; St.4: Sampling station Korucuk; St.5: Sampling station Sigma.

Table 1

Cd, Cr, Cu and Pb analysis results of water and sediment samples taken from sampling stations 1–5. Metal concentrations of water and sediment samples are given as μg/l and μg/g or mg/g dry weight, respectively. Data are presented as the mean ± SD of triplicate measurements

Sampling Stations	Sampling Times	[Cd], (μg/l)	[Cd], (μg/g dry weight)	[Cr], (μg/l)	[Cr], (mg/g dry weight)	[Cu], (μg/l)	[Cu], (μg/g dry weight)	[Pb], (μg/l)	[Pb], (μg/g dry weight)
Saricay	Mar–May 07	1.29 ± 0.45	**	24.53 ± 1.40	**	9.24 ± 0.59	**	5.00 ± 1.01	**
	Jun–Aug 07	1.43 ± 0.30	***	26.31 ± 0.99	1.00 ± 0.03	*	26.58 ± 1.66	2.86 ± 0.00	36.25 ± 2.02
	Sep–Nov 07	2.12 ± 0.54	***	27.30 ± 0.53	3.95 ± 0.85	*	14.29 ± 1.77	4.29 ± 0.00	16.49 ± 4.57
	Dec 07–Feb 08	*	***	28.64 ± 0.40	3.17 ± 0.92	0.50 ± 0.09	10.94 ± 2.58	*	8.29 ± 2.24
	Mar–May 08	0.67 ± 0.60	***	7.56 ± 0.27	1.38 ± 0.12	0.65 ± 0.13	9.45 ± 0.35	*	11.90 ± 1.35
	Jun–Aug 08	1.34 ± 0.66	***	10.61 ± 0.34	1.40 ± 0.25	0.64 ± 0.30	13.22 ± 6.39	*	21.06 ± 9.64
Curuksu	Mar–May 07	1.05 ± 0.22	**	32.98 ± 0.35	**	41.45 ± 1.02	**	*	**
	Jun–Aug 07	0.93 ± 0.05	***	29.02 ± 0.24	2.00 ± 0.00	37.53 ± 0.63	10.29 ± 0.00	11.43 ± 0.00	11.25 ± 0.00
	Sep–Nov 07	*	***	32.46 ± 0.47	2.71 ± 0.03	20.50 ± 2.97	19.85 ± 2.09	*	29.79 ± 7.64
	Dec 07–Feb 08	*	***	36.07 ± 0.68	1.47 ± 1.10	1.04 ± 0.13	16.57 ± 3.11	7.85 ± 3.03	28.21 ± 3.01
	Mar–May 08	0.39 ± 0.30	***	13.22 ± 0.39	0.70 ± 0.14	5.64 ± 0.66	18.36 ± 2.03	*	20.63 ± 3.13
	Jun–Aug 08	*	***	11.74 ± 0.40	1.22 ± 0.60	3.56 ± 0.18	23.02 ± 12.03	*	22.74 ± 10.50
Guzelkoy	Mar–May 07	1.02 ± 0.36	**	31.25 ± 0.43	**	45.44 ± 1.65	**	*	**
	Jun–Aug 07	0.79 ± 0.40	3.38 ± 0.53	28.99 ± 0.33	0.65 ± 0.25	26.67.840 ± 0.56	21.33 ± 7.04	1.43 ± 0.20	22.32 ± 12.12
	Sep–Nov 07	*	2.83 ± 0.52	29.50 ± 0.18	3.49 ± 0.81	19.20 ± 3.67	14.71 ± 1.77	2.14 ± 1.01	15.24 ± 5.38
	Dec 07–Feb 08	1.06 ± 0.41	5.00 ± 0.25	29.89 ± 0.61	2.81 ± 1.66	6.33 ± 2.26	11.20 ± 3.91	*	17.71 ± 0.29
	Mar–May 08	0.58 ± 0.20	3.00 ± 0.00	13.26 ± 0.44	0.96 ± 0.23	2.03 ± 0.34	20.54 ± 3.98	*	19.63 ± 0.38
	Jun–Aug 08	0.60 ± 0.31	4.17 ± 1.81	17.11 ± 0.47	1.20 ± 0.50	8.77 ± 0.55	38.00 ± 23.50	*	28.14 ± 10.15
Korucuk	Mar–May 07	0.85 ± 0.91	**	40.65 ± 0.57	**	43.75 ± 0.81	**	*	**
	Jun–Aug 07	*	4.00 ± 1.77	25.82 ± 0.55	0.46 ± 0.58	25.21 ± 0.99	7.02 ± 3.08	2.86 ± 0.11	3.04 ± 0.00
	Sep–Nov 07	2.40 ± 0.68	4.42 ± 0.95	28.77 ± 0.54	2.44 ± 2.07	16.40 ± 2.41	11.26 ± 3.49	2.14 ± 1.01	12.95 ± 7.10
	Dec 07–Feb 08	1.44 ± 0.41	3.75 ± 1.51	30.74 ± 0.36	2.01 ± 0.99	1.32 ± 0.27	10.33 ± 3.62	*	21.52 ± 0.88
	Mar–May 08	0.63 ± 0.36	4.50 ± 0.00	17.22 ± 0.50	0.51 ± 0.27	2.78 ± 0.34	8.68 ± 4.70	*	19.38 ± 0.63
	Jun–Aug 08	0.35 ± 0.25	3.92 ± 0.52	23.36 ± 0.67	0.25 ± 0.10	5.32 ± 0.32	3.11 ± 2.91	*	23.15 ± 4.26
Sigma	Mar–May 07	0.77 ± 0.22	**	21.72 ± 0.37	**	22.97 ± 1.02	**	*	**
	Jun–Aug 07	0.29 ± 0.41	4.63 ± 1.94	24.34 ± 0.24	0.33 ± 0.11	0.80 ± 0.00	10.84 ± 3.55	4.29 ± 2.02	17.99 ± 8.78
	Sep–Nov 07	1.06 ± 0.41	4.50 ± 0.90	27.41 ± 0.37	2.12 ± 1.39	9.30 ± 4.95	18.39 ± 6.05	*	25.18 ± 5.41
	Dec 07–Feb 08	0.40 ± 0.30	4.50 ± 0.76	29.05 ± 0.46	1.58 ± 0.76	4.43 ± 0.40	13.96 ± 3.12	*	26.74 ± 1.74
	Mar–May 08	2.05 ± 0.47	3.13 ± 0.45	12.54 ± 0.46	0.57 ± 0.09	3.80 ± 0.33	16.71 ± 9.39	*	21.25 ± 1.25
	Jun–Aug 08	1.03 ± 0.72	4.83 ± 0.58	15.02 ± 0.69	0.78 ± 0.30	0.86 ± 0.33	15.29 ± 10.48	*	20.57 ± 2.68

*Data were not included as they were not determined.

**Data were not included as they were not sampled.

***Data were not included as they were not measured.

diode array detector; Shimadzu CTO-20A column oven; Inertsil ODS-3 5 µm, 4.6 I.D. × 250 mm colon) using Supelco PAH Calibration mixture as standard. For this purpose, 500 ml of water sample was loaded onto a LC18 SPE cartridge previously washed with 5 ml of water-methanol (1:1, v/v) and dried completely by means of a vacuum. Arrested substances were eluted with 5 ml hexane. The collected eluent had been dried down and reconstituted with 500 µl acetonitrile before analyzing with HPLC.

2.4. Measurement of enzyme activities

Oligochaete *L. profundicola* was collected from three sampling stations (Guzelkoy, Korucuk and Sigma) of Menderes River and transferred to laboratory for biomarker analysis. Homogenization and preparation of cytosolic and microsomal fractions performed according to Schenkman and Cinti [33] as optimized by Ozkarsli et al. [34]. Aliquots of fractions were stored at –80 °C for biomarker analysis. Protein concentrations of cytosolic and microsomal fractions were determined by the method of Lowry et al. [35].

Microsomal EROD activities were assayed by the methods of Burke and Mayer [36] as optimized by Arinc and Sen [37] using 7-ethoxyresorufin as a substrate on Cary Eclipse fluorometer. MT contents of *L. profundicola* were determined by the method of Viarengo et al. [4] and as optimized Ozdemir et al. [38].

2.5. Statistical analysis

Seasonal variations of heavy metal concentrations in the sediment and EROD activities and MT contents of *L. profundicola* were analyzed by one way analyses of variance (one way ANOVA) and the two sample-*t* test.

3. Results and discussion

In this study, Cd, Cr, Cu and Pb metal analysis of water and sediment samples and PAH concentrations of water samples of Saricay, Curuksu, Guzelkoy, Korucuk and Sigma stations along Curuksu stream on Menderes River were investigated throughout 20 mon to detect to metal and PAH contamination. As seen in Table 1, Cu, Pb and Cd metals were detected as µg/g dry weight in sediment samples of five sampling stations while Cr concentrations were detected as mg/g dry weight. Analysis of Cu, Cr, Cd and Pb metals in water and sediment samples were showed seasonal variations ($p < 0.0001$). For instance, Cu concentrations of sediment samples are higher in summer season while Cd is higher in summer and winter. In addition to these heavy metals, naphthalene, acenaphtylene, acenaphtene, phenanthrene, anthracene, pyrene and chrysene were also found in water samples. Fig. 2 shows the variation of total PAH concentrations in water samples throughout 20 mon. As seen in Fig. 2 the total PAH concentrations of water samples from Curuksu and Saricay were significantly higher in 2007.

In addition to chemical analysis, EROD activities and MT contents of *L. profundicola* collected from Guzelkoy, Korucuk and Sigma sites along Curuksu stream on Menderes River were also studied. As seen in Fig. 3, EROD activities of *L. profundicola* were induced in some seasons and these inductions were showed some similar variations with total PAH analysis results. Also, our

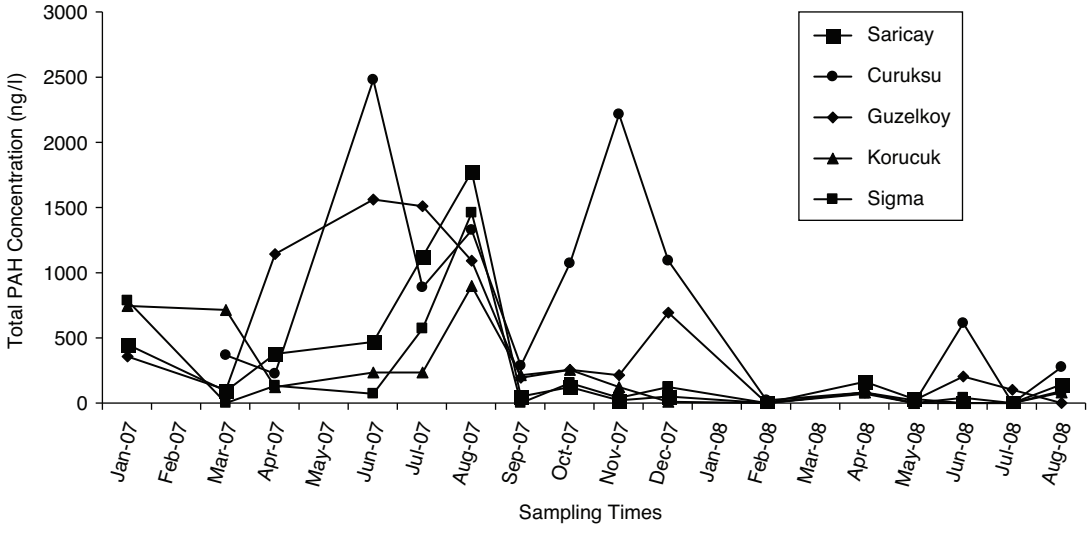

Fig. 2. PAH analysis of water samples taken from sampling stations 1–5.

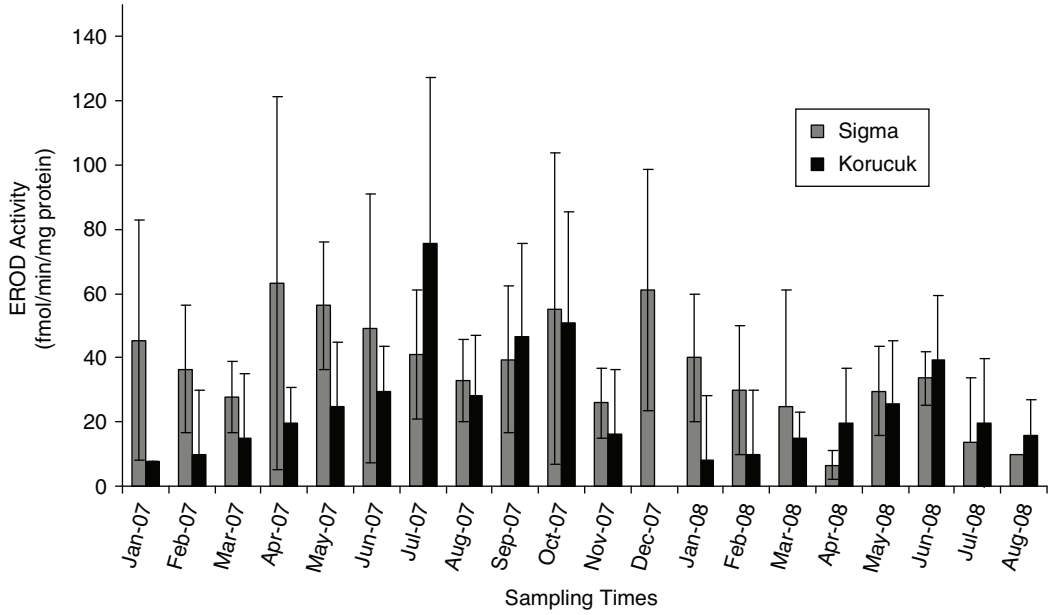

Fig. 3. EROD activities of *L. profundicola* collected from sampling stations 4 and 5. Data are presented as the mean ± SD of at least three sets of triplicate measurements.

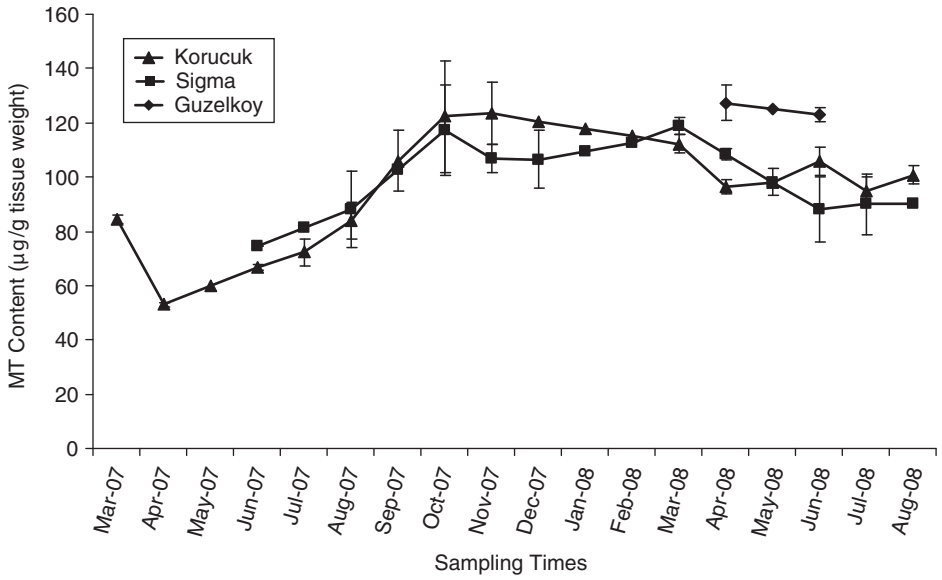

Fig. 4. MT contents of *L. profundicola* collected from sampling stations 3, 4 and 5. Data are presented as the mean ± SD of at least three sets of triplicate measurements.

previous biomarker studies using *L. profundicola* as a bioindicator organism were showed the PAH contamination and induced EROD activities in Curuksu stream on Menderes River [38]. Fig. 4 shows MT contents of *L. profundicola* collected from Guzelkoy, Korucuk and Sigma stations. As seen in Fig. 4, MT contents of *L. profundicola* were found to be escalating at the beginning of spring 2007 and reaching to highest level in October 2007. Furthermore, it stayed at around the same level for year 2008. It suggested that the low level of precipitation and arid conditions might have caused increased MT levels in samples in year 2007. These variations in MT contents of *L. profundicola* were found to be statistically significant ($p < 0.001$). Similar results were also obtained in heavy metal analysis and our correlation results were showed MT measurements are quite in good accordance

with metal analysis of sediment samples which confirmed metal contamination in Curuksu stream. Similarly, Akcay et al. [39], Turgut [40] and Koca et al. [41] have reported heavy metal pollution from agricultural, industrial or domestic waste discharges in Menderes River.

4. Conclusion

In conclusion, PAH and heavy metal pollutions in Curuksu Stream on Menderes River were investigated by chemical analysis and biomarker studies. Our results showed that Curuksu and Guzelkoy stations where industrial and domestic discharges exist excessive are found to be more than Korucuk and Sigma. In addition, Saricay was the least polluted area among the sampling stations.

Acknowledgements

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