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The Study of the Genotoxic Effect of the Karasu River Surface Water Containing Local Fat Plant Waste Water on the *Drosophila melanogaster* Using the Wing Somatic Mutation and Recombination Test (SMART)

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Abstract

In the present study, the genotoxic effect of wastewater including heavy metals was investigated. For this purpose, the induction/reduction of mutation and recombination of surface water sampled before and after the discharge points (station-I,II) of the local fat plant (Erzurum, Turkey) were investigated by wing Somatic Mutation and Recombination Test (SMART) of the *Drosophila melanogaster*. In this study, third-instar larvae, which are trans-heterozygous for the third chromosome recessive markers with multiple wing hairs (*mwh*) and flare-3 (*flr*^{3}) were separated into groups and were exposed to two different water samples including high and low metal ion concentrations. These larvae were kept in the culture media until the adult stage. Wing preparates of obtained adult individuals were prepared and examined under a light microscope. Frequencies of mutant clones formed through application of surface water samples taken from station I and station II in different periods and frequencies of the control group were compared by a Conditional Binominal Test. While it was observed that the samples taken from station I did not cause an increase in the total mutant clone frequency whereas the samples obtained from station II did. It was suggested that the metal ion concentration was determined to be higher in station II which might have lead to this result.

Keywords: *Drosophila melanogaster*, fat plant, genotoxicity, SMART, wastewater,

Yerel Yağ Fabrikası Atık Suyunu İçeren Karasu Nehri Yüzey Suyunun Genotoksik Etkisinin *Drosophila melanogaster* Kanat Somatik Mutasyon ve Rekombinasyon Testi (SMART) ile Araştırılması

Özet

Sunulan çalışmada, ağır metal içeren atık suların genotoksik etkisi araştırıldı. Bu amaçla, *Drosophila melanogaster*`in kanat Somatik Mutasyon ve Rekombinasyon Testi (SMART) ile yerel yağ fabrikasının (Erzurum, Türkiye) atıklarının döküldüğü noktanın öncesinde ve sonrasında (istasyon I-II) yüzey su örneklerinin mutasyon ve rekombinasyonunu artırıp artırmadığı araştırıldı. Bu çalışmada, üçüncü kromozomları üzerinde çoklu kıl (*mwh*) ve topaklaşmış kıl (*flr*^{3}) meydana getiren iki farklı çekinik işaret gen taşıyan trans-heterozigot üçüncü evre larvalar gruplara ayrılarak, yüksek ve düşük metal konsantrasyonlarını içeren iki farklı su örneği uygulandı. Bu larvalar ergin oluncaya kadar bu kültür ortamlarında tutuldu. Elde edilen ergin bireylerin kanat preparatları hazırlanarak ışık mikroskobunda incelendi. Binominal Şartlı Test kullanılarak istasyon I ve istasyon II'den farklı dönemlerde alınan yüzey su örneklerinin uygulanması ile oluşan mutant klonların frekansları, kontrol grubu frekansları ile karşılaştırıldı. İstasyon I'den alınan örneklerin toplam mutant klon frekansında artışa neden olmadığı gözlenirken istasyon II'den alınan örneklerin ise mutant klon frekansını arttırdığı bulunmuştur. İstasyon II'de daha yüksek olduğu belirlenen metal iyon konsantrasyonunun bu sonuca neden olabileceği öne sürülmüştür.

Anahtar Kelimeler: Atık su, *Drosophila melanogaster*, genotoksisite, SMART, yağ fabrikası

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INTRODUCTION

Today, heavy metals are plentiful in our drinking water, air, and soil. They are present in virtually every area of modern consumerism from

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construction materials to cosmetics, medicines to processed foods, fuel sources to agents of destruction, and appliances to personal care products. The degree to which a system, cell, tissue, or organ is affected by a heavy metal toxin depends on the toxin itself and the living organisms degree of exposure to the toxin (Baldwin and Marshal 1999).

Water pollution has also become an international problem. Even countries with little industry have reason to be alarmed by this appalling situation. Industries and individuals dump waste materials into rivers, oceans, and even local water supplies. This situation affects living organisms negatively (White and Rasmussen 1998).

Karasu River is located in the upper Euphrates River Basin in eastern Turkey (Erzurum province). The Karasu River which is used for farming and drinking water is polluted not with only industrial waste waters but also domestic ones. The local fat plant (Doyasan - Erzurum, Turkey) (Figure 1) works as a butter factory during the summer season and wastages are drained into the Karasu River without any filtering process.

Studies of the genotoxicity can help to evaluate the safety and effectiveness of environmental samples. Of these, the SMART on the *Drosophila melanogaster* wings (Graf et al. 1984) is used to find the genotoxicity of various environmental samples (Delgado-Rodriguez et al. 1999, Amaral et al. 2005, Pantaleao et al. 2007, Pimenta et al. 2008). Because this process is inexpensive and the detection of genotoxic damage takes only a short time (approximately ten days). The SMART is also used in Turkey in order to assess the impact of pollution (Yeşilada 2001, Sarıkaya and Çakır 2005). In the present study, we have used the SMART to evaluate the genotoxicity of the surface water of the Karasu River.

MATERIALS AND METHODS

Sample sites and water collection

Water samples were taken from two stations (one 50 m before the discharge point of the fat plant and the other 50 m after the discharge point of the fat plant) (Figure 1) monthly (Table 1) in the summer season of 2007 and various water quality parameters (pH, dissolved oxygen and temperature) were measured at the site by a portable multi-parameter (WTW multiline P-4 F SET-3).

Physicochemical analysis of water samples

Total hardness, chloride and phosphate measurements were made according to Standard Methods (Anonymous 1985). Total organic matter levels were determined as total organic carbon by a TOC Analyzer (Teledyne-Tekmar Apollo 9000). Metal ions (Fe, Cu, Mn, Zn, Pb, Cd) were measured by an Atomic Absorption Spectrophotometer (Perkin-Elmer). The analytical determination of boron was done potentiometrically by means of mannitol, which forms a complex compound with boric acid. For this purpose, the boron analysis was carried out as follows: the solution pH was adjusted to 7.60 after the sample was filtered and then 5 g mannitol was then added to the solution. The solution was titrated with 0.5 N KOH until the solution pH was 7.60 (Yılmaz et al. 2005).

Negative and positive controls

Pure distilled water served as a negative control. A 1 mM aqueous solution of ethyl methanesulfonate (EMS) was used as the positive control. EMS was purchased from Merc (Darmstadt, Germany) and was reagent grade.

Somatic mutation and tecombination test (SMART)

Two *D. melanogaster* strains carrying markers on the left arm of chromosome 3 were used: (i) flare (*flr*^{3}, 3-38.8) and (ii) multiple wing hairs (*mwh*, 3-0.3). For more detailed information on the genetic symbols and descriptions see Lindsley and Zimm (1992). The strains were obtained from the Genetic Research Laboratory, University of Akdeniz in Turkey. Eggs derived from the standard cross flare virgin females crossed with *mwh* males were collected for 8 h on a standard medium enriched with baker's yeast (Graf and van Schaik 1992). Three days later, the larvae from the standard cross, were transferred to vials containing 4.5 g of a dry *Drosophila* instant medium (Carolina Biological Supply, Burlington, NC, U.S.A.) dehydrated with 9 ml of the test solutions. Negative solvent controls were always included. The larvae were allowed to feed on the media until the adult stage (approx. 6 days). Emerged adult flies of the two genotypes namely marker-heterozygous (*mwh/flr*^{3}=MH) and balancer heterozygous (*mwh/TM3*, Bds=BH) were collected and stored in 70% ethanol. Their wings were mounted in Faure's solution (gum arabic 30 g, glycerol 20 ml, chloral hydrate 50g and water 50ml) and inspected under 400× magnification for the presence of mutant spots. The number of spots as

Table 1. Physico-chemical parameters of station I, II.

		PARAMETERS														
Sampling date		t (°C)	pH	DO (mg/L)	Ch (mg/L)	P (mg/L)	TDS (mg/L)	C (mg/L)	H (mg $CaCO_3$/L)	B (µg/L)	Fe (µg/L)	Cu (µg/L)	Zn (µg/L)	Mn (µg/L)	Pb (µg/L)	Cd (µg/L)
STATION-I	Jun.	21.6	8.09	8.10	51.00	-	370	1.43	94.20	1.0622	71.6	0.71	3.1	3.24	0.43	2.8
	July.	24.4	8.31	8.00	81.20	-	730	2.50	151.8	0.3171	77.0	1.25	3.6	3.04	0.95	0.5
	Aug.	21.0	7.96	9.35	70.40	-	660	1.50	142.0	0.3700	68.0	0.89	3.8	3.38	0.53	0.1
	Sep.	17.5	8.10	5.50	32.90	-	495	4.13	123.6	1.1706	65.2	1.07	4.1	2.70	0.45	3.8
	Oct.	15.0	7.95	6.50	44.53	-	421	2.80	110.4	0.6589	71.2	1.32	3.4	2.97	0.97	1.6
STATION-II	Jun.	22.1	7.56	1.10	82.00	-	535	3.72	104	1.1679	66.7	2.50	5.4	7.52	3.63	6.6
	July.	22.7	7.47	2.10	69.00	0.50	725	1.93	99.2	0.2960	68.0	1.79	4.6	3.26	4.35	1.4
	Aug.	21.0	7.53	0.80	84.30	0.86	633	2.33	102.2	0.7729	64.9	2.68	5.0	3.90	3.49	3.8
	Sep.	16.0	7.61	1.00	43.20	-	570	6.77	104.0	1.5326	60.0	3.40	4.3	7.02	3.88	7.1
	Oct.	14.0	7.50	1.03	51.63	-	560	3.50	103.5	0.9645	67.9	2.76	4.9	5.86	4.15	2.8

* t: Temperature (°C); DO: Dissolved Oxygen(saturation %); Ch: Chloride Concentration; P: Phosphate Concentration; TDS: Total Dissolved Solids; C: Carbon Concentration; H: Hardness; B: Boron Concentration; Fe: Iron Concentration; Cu: Copper Concentration; Zn: Zinc Concentration; Mn: Manganese Concentration; Pb: Lead Concentration; Cd: Cadmium Concentration

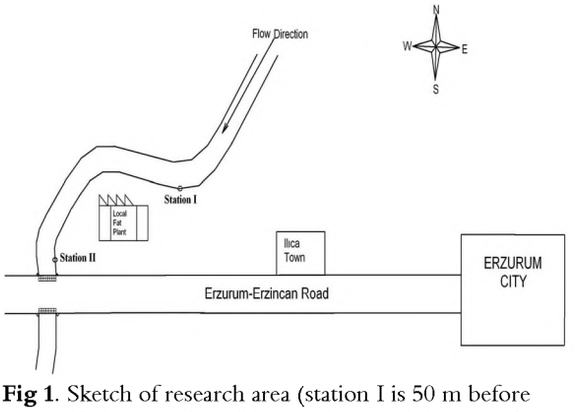

Fig 1. Sketch of research area (station I is 50 m before discharge point of fat the plant, and the other is 50 m after discharge point of the fat plant).

well as their type and size were recorded. A comparison of the results obtained from MH and BH flies were used to measure the mutagenic and recombinogenic potential of the test samples (Amaral et al. 2006, Silva et al. 2006).

On the marker-heterozygous wings, two types of spots could be observed: (i) single spots, either *mwh* or *flr*^{3}, produced by somatic point mutation, chromosome aberration as well as mitotic recombination; (ii) twin spots, consisting of both *mwh* and *flr*^{3} sub clones, originated exclusively from mitotic recombination (Graf et al. 1984). On the balancer-heterozygous wings, *mwh* single spots reflect predominantly somatic point mutation and chromosome aberration, since products of mitotic recombination involving the multiple inverted balancer chromosome (*TM3*) and its structurally normal homologue are normally non-viable (Szabad et al.1983).

Statistical analysis

For the evaluation of the genotoxic effects recorded, the frequencies of each type of spot per fly of a treated series were compared to its concurrent negative (solvent) control series. These statistical comparisons were done using the Kastenbaum-Bowman (1970) test for proportions and followed the multiple-decision procedure according to Frei and Wurgler (1988).

RESULTS AND DISCUSSION

The various water quality parameters (pH, dissolved oxygen, temperature, and some heavy metal ions) are shown in Table I. The results obtained from the two sites in the wing SMART assay after the chronic exposure of larvae from the standard cross is shown in Table II and Table III, where the spot data is given for the MH (*mwh/flr*^{3}) and BH (*mwh/TM3*) genotypes.

According to Table I, some heavy metal ion concentrations (Cu, Zn, Mn, Pb, and Cd) of station II were higher than station I respectively. For example, in June, while Cd concentration of station I was 2.8, it was 6.6 for station II. Similarly, Mn, Zn, and Cu concentrations also distinctly increased at station II in August. In the other two heavy metal ion concentrations (B and Fe), a serious difference was not observed in stations I and II.

Statistically significant increases in the total spots in the MH generations were determined for each month in station II. However, station I demonstrated inconclusive results for total spots except for the October (small single spots, large single spots, and twin spots were inconclusive, but total *mwh* spots and total spots were positive) samples (Table II). For BH flies, station I samples showed negative and inconclusive results while station II demonstrated positive results for total spots in all samplings (Table III).

According to these results, it was determined

Table 2. Summary of results obtained with the *Drosophila* Somatic Mutation and Recombination Test (SMART) after the chronic treatment of larvae from MH individuals with surface water samples from the Karasu River.

Cross sampling time and type of descendent scored (genotype)	Treatment/ site of collection	Number of wings (N)	Spots per fly (no. of spots)/statistical diagnosis[a]														
			Small single spots (1-2 cells) (m=2)			Large single spots (>2 cells) (m=5)			Twin spots (m=5)			Total mwh spots (m=2)			Total spots (m=2)		
			No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D
June 2007 MH (*mwh/flr*^{3})	Control	80	13	(0.16)		1	(0.01)		0	(0.00)		14	(0.18)		14	(0.18)	
	1 mM EMS	80	20	(0.25)	i	12	(0.15)	+	9	(0.11)	+	32	(0.40)	+	41	(0.51)	+
	Station I	80	18	(0.23)	i	3	(0.04)	i	1	(0.01)	i	21	(0.26)	i	22	(0.28)	i
	Station II	80	32	(0.40)	+	6	(0.08)	i	6	(0.08)	+	38	(0.48)	+	44	(0.55)	+
July 2007 MH (*mwh/flr*^{3})	Control	80	15	(0.19)		1	(0.01)		0	(0.00)		16	(0.20)		16	(0.20)	
	1 mM EMS	80	45	(0.56)	+	5	(0.06)	i	10	(0.13)	+	50	(0.63)	+	60	(0.75)	+
	Station I	80	23	(0.29)	i	3	(0.04)	i	0	(0.00)	i	26	(0.33)	i	26	(0.33)	i
	Station II	80	58	(0.73)	+	7	(0.09)	+	8	(0.10)	+	65	(0.81)	+	73	(0.91)	+
August 2007 MH (*mwh/flr*^{3})	Control	80	14	(0.18)		3	(0.04)		1	(0.01)		17	(0.21)		18	(0.23)	
	1 mM EMS	80	54	(0.68)	+	19	(0.24)	+	41	(0.51)	+	73	(0.91)	+	114	(1.43)	+
	Station I	80	20	(0.25)	i	0	(0.00)	-	0	(0.00)	i	20	(0.25)	i	20	(0.25)	i
	Station II	80	37	(0.46)	+	8	(0.10)	+	6	(0.08)	+	45	(0.56)	+	51	(0.64)	+
September 2007 MH (*mwh/flr*^{3})	Control	80	12	(0.15)		0	(0.00)		1	(0.01)		12	(0.15)		13	(0.16)	
	1 mM EMS	80	51	(0.64)	+	28	(0.35)	+	15	(0.19)	+	79	(0.99)	+	94	(1.18)	+
	Station I	80	18	(0.23)	i	3	(0.04)	i	1	(0.01)	i	21	(0.26)	i	22	(0.28)	i
	Station II	80	42	(0.53)	+	6	(0.08)	+	3	(0.04)	i	48	(0.60)	+	51	(0.64)	+
October 2007 MH (*mwh/flr*^{3})	Control	80	10	(0.13)		2	(0.03)		0	(0.00)		12	(0.15)		12	(0.15)	
	1 mM EMS	80	31	(0.39)	+	9	(0.11)	+	6	(0.08)	+	40	(0.50)	+	46	(0.58)	+
	Station I	80	15	(0.19)	i	8	(0.10)	i	1	(0.01)	i	23	(0.29)	+	24	(0.30)	+
	Station II	80	29	(0.36)	+	11	(0.14)	+	1	(0.01)	i	40	(0.50)	+	41	(0.51)	+

aStatistical diagnoses according to Frei and Würgler [1988, 1995] for comparison of response with corresponding negative control: -, negative; i, inconclusive; +, positive ($P < 0.05$);
m, minimal risk multiplication factor for the assessment of negative results; MH, marker-heterozygous.

Table 3. Summary of results obtained with the *Drosophila* Somatic Mutation and Recombination Test (SMART) after the chronic treatment of larvae from BH individuals with surface water samples from the Karasu River.

Cross sampling time and type of descendent scored (genotype)	Treatment/ site of collection	Number of wings (N)	Spots per fly (no. of spots)/statistical diagnosis[a]														
			Small single spots (1-2 cells) (m=2)			Large single spots (>2 cells) (m=5)			Twin spots (m=5)			Total mwh spots (m=2)			Total spots (m=2)		
			No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D	No	Fr	D
June 2007 BH (*mwh/TM3*)	Control	80	17	(0.21)		3	(0.04)		[b]			20	(0.25)		20	(0.25)	
	1 mM EMS	80	33	(0.41)	+	22	(0.28)	+				55	(0.69)	+	55	(0.69)	+
	Station I	80	18	(0.23)	-	4	(0.05)	i				22	(0.28)	-	22	(0.28)	-
	Station II	80	37	(0.46)	+	9	(0.11)	i				46	(0.58)	+	46	(0.58)	+
July 2007 BH (*mwh/TM3*)	Control	80	14	(0.18)		3	(0.04)					17	(0.21)		17	(0.21)	
	1 mM EMS	80	49	(0.61)	+	38	(0.48)	+				87	(1.09)	+	87	(1.09)	+
	Station I	80	16	(0.20)	i	5	(0.06)	i				21	(0.26)	i	21	(0.26)	i
	Station II	80	52	(0.65)	+	8	(0.10)	i				60	(0.75)	+	60	(0.75)	+
August 2007 BH (*mwh/TM3*)	Control	80	14	(0.18)		0	(0.00)					14	(0.18)		14	(0.18)	
	1 mM EMS	80	61	(0.76)	+	15	(0.19)	+				76	(0.95)	+	76	(0.95)	+
	Station I	80	24	(0.30)	i	1	(0.01)	i				25	(0.31)	i	25	(0.31)	i
	Station II	80	40	(0.50)	+	6	(0.08)	+				46	(0.58)	+	46	(0.58)	+
September 2007 BH (*mwh/TM3*)	Control	80	13	(0.16)		2	(0.03)					15	(0.19)		15	(0.19)	
	1 mM EMS	80	32	(0.40)	+	19	(0.24)	+				51	(0.64)	+	51	(0.64)	+
	Station I	80	20	(0.25)	i	5	(0.06)	i				25	(0.31)	i	25	(0.31)	i
	Station II	80	46	(0.58)	+	9	(0.11)	+				55	(0.69)	+	55	(0.69)	+
October 2007 BH (*mwh/TM3*)	Control	80	16	(0.20)		4	(0.05)					20	(0.25)		20	(0.25)	
	1 mM EMS	80	34	(0.43)	+	40	(0.50)	+				74	(0.93)	+	74	(0.93)	+
	Station I	80	19	(0.24)	i	1	(0.01)	-				20	(0.25)	-	20	(0.25)	-
	Station II	80	49	(0.61)	+	16	(0.20)	+				65	(0.81)	+	65	(0.81)	+

[a]Statistical diagnoses according to Frei and Würgler [1988, 1995] for comparison of response with corresponding negative control: -, negative; i, inconclusive; +, positive ($P < 0.05$);
m, minimal risk multiplication factor for the assessment of negative results; BH, balancerheterozygous.
[b]Balancer chromosome TM3 does not carry the flr3 mutation.

that waste waters including heavy metals (station II) have a genotoxic effect on *Drosophila*. Similar results have also been observed by other researchers. Pantaleao et al. (2007), reported the impact of pollution on the Japaratuba River in Brazil using the *Drosophila* wing spot test. In addition, Pimenta et al. (2008), have also investigated genotoxicity of water from the Paraguay River near Caceres-MT, Brazil by *Drosophila* wing spot test. Amaral et al. (2005), determined the genotoxicity of surface waters collected from the Cai River (Rio Grande do Sul, Brazil). These findings are in accordance with our results. Although mitotic recombination was involved in these responses, the genotoxicity of these samples was partially due to mutational events. As was the case with some of our results, the genotoxicity of these water samples was observed in the MH flies.

It is known that heavy metals are poisonous for living organisms by damaging cellular enzymes. It was stated that the genotoxic damages are arising from the co-efficient of high heavy metal ion concentrations in the *Drosophila melanogaster*.

It is very difficult for anyone to avoid exposure to any of the many harmful heavy metals that are so prevalent in our environment. The present study may help to lessen the negative impact that these agents have on human health.

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