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Acute and Chronic Toxicity of Contaminated Fresh Water and Sediment of Nif Brook on ***Daphnia magna*** (Straus, 1820)

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Özet: ***Nif Çayi Kirletilmiş Tatli Su ve Sedimentinin*** Daphnia magna ***(Strauss, 1820) Üzerine Akut Ve Kronik Toksisitesi.*** Nif Çayı Endüstriyel, evsel ve tarımsal deşarjlar ile doğrudan kirlenmektedir. Çayın suyunun evsel ve endüstriyel alanlarda sulama ve yıkama suyu olarak kullanılması rahatsızlık verici boyuttadır, bu nedenle doğal populasyonlar için potansiyel toksisitesinin bilinmesi büyük öneme sahiptir. Bu çalışmanın amacı Nif Çayı su ve sediment örneklerinin su piresi *Daphnia magna* kullanılarak toksisitesinin belirlenmesidir. Su ve sediment örneklerinin letal toksisitesi 48-saat akut test, subletal toksisite ise 7-gün kronik test ile gerçekleştirilmiştir. *D.magna* kullanılarak yapılan 48 saat Akut test sonuçlarına göre LC 50 değerleri su örnekleri için 6.8 ile 12.67 µl/L arasında sediment örnekleri için 6.826 ile 38.038 µg/L arasında bulunmuştur. Subletal konsantrasyonlara maruz bırakılarak yapılan kronik testler sonucunda tüm istasyonlardan alınan su ve sediment örneklerin canlının üremesi üzerine negatif etkisi olduğu gözlenmiştir. Elde edilen veriler ışığında bu deneme sisteminin rutin kirlilik belirleme çalışmaları için uygunluğu tespit edilmiştir.

Anahtar Kelimeler: Nif Çayı, *Daphnia magna*, Akut toksisite, Kronik toksisite.

Abstract: The water and sediment of Nif Brook (Izmir, Turkey) is polluted by effluents have been discharged directly or treated from industrial, domestic and agricultural sources. As the water of the brook used for domestic and industrial water supply as well as for purpose of irrigation, it has a great importance to know the toxicity potential on the natural populations. The aim of this study is to define the water and sediment toxicity of certain streams Nif Brook by using the water flea Daphnia magna Strauss as a test organism. The toxic contents of collected water were diluted and sediments were used as an extract. The lethal toxicity of waters and sediments were evaluated by using the 48- h acute toxicity test. And also sub-lethal toxicity of waters and sediments were evaluated by using 7-days chronic toxicity test. According to acute test results average 48 h LC50 for *D.magna* between 6.8 to 12.67 µl/L for water samples, between 6.83 to 38.0 µg/L for sediment samples of 4 stations in Nif Brook were found and then *D.magna* were exposed to sub-lethal concentrations of 4 stations in this stream, the results show that all of the streams and their elutriates produced negative effects. Results of this study showed that, screening the toxicity of polluted waters and sediments by using *D.magna* gives better and more meaningful results than an quantitative analyze of toxic matters.

Key Words: Nif Brook, *Daphnia magna*, Acute Toxicity, Chronic Toxicity

Introduction

Daphnia magna is one of the most important fresh water species employed in ecotoxicity testing through the world. Except for fish and more recently algae, chronic and acute tests with *D.magna* are among the most frequently performed studies in aquatic toxicology (OECD 2004). *D.magna* has been used extensively to determine the toxicity of effluents, water and sediment samples and has been demonstrated to be sensitive to many environmental contaminants. The choice of *D.magna* for use as a standard test species was strongly influenced by the following factors: reproduction is normally parthenogenic, which allows the maintenance and testing of clones; it can be cultured in the laboratory; it represents the zooplankton community, a major element of the freshwater food chain; as a species of worldwide occurrence, the ecological relevance of the test results is recognized. Daphnids are important invertebrate species in aquatic food webs. Most daphnids are cyclic partheogenetic species capable of both asexual and sexual reproduction. Laboratory cultures of Daphnids are typically maintained in partenogenetic state.

The objective of present study was to examination of Nif Brook water and sediment quality in Izmir, Turkey. Kemalpasa region and Nif Brook are close to Izmir city located near the Aegean coastline of Turkey and have been polluted intensively as a consequence of significant increases in population, and numerous industrial facilities and investments (Kucuksezgin et al., 2008, Arslan et al., 2009a). Such streams as Nif Brook passing through urban areas receive chemical discharges from industrial, municipal, and agricultural sources. The water of Nif Brook is used for domestic and industrial water supply and for purpose of irrigation. There are 138 industrial facilities in the region involved in such industrial fields as food, machine, rubber, plastic and other petrochemical products, chemicals, textile, electric devices, households, beverage, leather and packing materials. 38 of these industrial facilities have treatment plant. Direct or

treated effluents from these sources have been discharged into Nif Brook (Anonymous 2001). As Furlong et al., (1988) had stated most of the discharged chemicals are hydrophobic in nature and sequestered by sediment particles that eventually settle in sediments. They may resist the microbial degradation and tend to accumulate in high concentrations in sediment or biota (Alexander 1981).The aim of this study was to measure the potential toxicity of water and sediment of Nif Brook.

In the world, aquatic toxicity test data are routinely used to evaluate risks associated with discharge of effluents into water bodies and sediments. Although analytical chemical testing methods give information regarding the quality and quantity of pollution, biological testing methods can give a qualitative description of the presence and strength of toxicity (Feiler et al., 2006). Chemicals present in environmental water and sediment as a complex mixture, so that their potential ecotoxicological effects are much complicated due to their interactions (Parlak et al., 2009). Ecotoxicology and concentration of those unknown chemicals in environmental water and sediment is undetectable. Therefore, it is quite difficult to evaluate the actual exposure levels and ecotoxicological effects of all coexisting chemicals on aquatic organisms by measuring concentrations of individual chemicals (U.S.EPA, 1990). Aquatic organisms of all trophic levels have been used in ecotoxicological evaluation of pollution in aquatic systems and sediments. Studies of aquatic ecosystems should therefore not be limited to the water column, but must also consider the sediment quality (Burton, 1991). Bioassays with sediments can be used to determine the bioavailabity of analyzed concentrations of contaminants in order to assess their pollutant potential. The toxicity of fresh water sediments has been assessed with the use of various fresh water invertebrates (Ingersoll et al. 1995). The Clodoceran *D. magna*, although not a sediment-dwelling organism, has shown its usefulness in evaluating fresh water sediment toxicity (Nebeker et al. 1984). The aims of this study is to define the acute and chronic toxicity of sediments and water from Nif Brook by using the water flea D.magna Straus as a test organism and to prove the appropriateness of this test system for routine studies to determine sediment and water toxicity.

Material and Methods

Sediment bioassays have usually been applied to sediments from sites close to known sources of pollution such as major industrial and agricultural sites. Sediment and water samples were collected from 5 stations located in Nif Brook in April 2007 (Figure 1).

The attentions were paid selecting the stations considering the locations around organized industrial zone (OIZ). The sediment samples were collected by grab sampler and placed in pre-cleaned jars and kept in ice-box until transferred to the laboratory.

Figure 1. Sampling stations on the Nif Brook.

Samples were air-dried, in room temperature and they stored in deep freeze at -20ºC until testing. Sediment samples from Nif Brook were sieved to powder and sifted in the laboratory and then placed in portions of 1g into sterile Teflon tubes and mixed with 1 ml hexane/chloroform/acetone (1:1:1 v:v:v) using a vortex mixer. Subsequently, the samples were centrifuged for 10 minutes at +4°C at 5600g (Sigma K3) and supernatants were transferred into sterile tubes. Supernatants was evaporated and then dissolved by adding 1 ml of dimethylsulfoxide (DMSO) (Kotelevtsev and Ludmila, 1995). The extracts of sediment samples were assayed in five different concentrations as 2.5, 5, 10 and 15 µg/L for acute toxicity test.

Water samples were used after filtrations through 0.45 µm and 0.20 µm filters. The water samples were tested by adding to the test medium in the ratio 10, 20, 40, 60, 80, 100 % (v:v) the control test series which include only test medium were also prepared. All of the test series had 4 replicate.

D.magna cultures consisted of glass beakers containing culture medium and 20 daphnids. Culture medium was renewed and offspring produced discarded twice weekly. Brood daphnids were discarded after 4 weeks in culture and replaced with neonatal organisms. Cultures were maintained at 21. 0.5°C under 18-h light:6-h dark photoperiod. The Daphnia were fed 75x106/ml cells the unicellular green algae Selenastrum copricornutum was cultured in Bristol medium. These culture conditions maintained the daphnids in the parteogenetic reproductive stage.

The primary objective of the 48-hour toxicity test using Daphnia magna. is to evaluate the acute toxicity of effluents, chemicals, and elutriates on freshwater crustaceans. The test

is conducted in small beakers or standard test tubes, and often incorporates replication (e.g., 5 organisms in each of 4 replicates). The measurement endpoints generally evaluated are the 48-hour LC50 (for survival), and the 48-hour EC50 (for immobility). The acute test was performed in accordance to the standard protocol for *D.magna* immobilization test (OECD 2004). 5 neonates aged less than 24 h, divided in to four groups were exposed to each concentration for 48 h in a static test. The test containers used were 20-ml glass beakers filled with 10 ml of test solution. The test was performed at 20 ± 2°C under 18-h light: 6-h dark photoperiod. Numbers of mobile and immobile specimens were registered after 24 and 48 h; pH and oxygen were measured in the controls and at the highest test concentrations. Daphnids were exposed to the following concentrations of sediment for 48-h: control, 2.5, 5, 10 and 15 µg/L and also daphnids were exposed to water samples at 10, 20, 40, 60, 80, 100 % (v: v).

The main objective of chronic toxicity tests to determine the effects of toxic substances on reproduction. For this reason, a chronic sub lethal level of toxic substances to be tested is very important. This is fundamentally acting in chronic experiments, as a result of acute tests acceptable limit of 10% during 48 hours of death was not observed concentrations are based on the lethal effect. LC50 values were 0.68, 0.27, 0.9, 12.6 µl/L at stations 1, 2, 3, 4 respectively from Nif Stream waters.. Acute LC50 values were calculated as a result of the test water and sediment samples obtained in chronic tests, LC50 values 1/10 used in calculating. Chronic lethal effect was not observed in the test concentrations for Stations 1, 2, 3 and 4 sediment samples, 0.68 µg/L, 3.8 µg/L, 0.98 µg/L and 21.2 µg/L concentrations of sediment samples consisted of. The effect of the water and sediment from 5 Stations in Nif Brook on the reproductive output was assessed in a semi-static test according to the standard protocol for *D.magna* Reproduction Test (OECD 1998). Daphnids, aged less than 11 day at the start of the test, was exposed for a period of 7 days. Each treatment consists of 200-ml beakers Each containing 100 ml test solution and a single test organism. The daphnia were fed 75x106/ml cells the unicellular green algae Selenastrum copricornutum was cultured in Bristol medium. Test solutions were renewed three times weekly. Survival and offspring production were assessed whenever solutions were renewed. Test was performed at 20±2°C under 18-h light: 6-h dark photoperiod. During the experiments, temperature, dissolved oxygen, pH, and total hardness were monitored weekly. Endpoints included adult survival, number of egg per individual and number of live neonates per individual. For this purpose the neonates were collected and counted every day.

The LC50's were calculated by probit analysis using Toxicologist 1.00 (1990) statistical software. All chronic data were tested for statistical significance using a single factor one-way analysis of variance (ANOVA).

Results

Water bioassay with daphnia 5 dilution was used (%10, 20, 40, 60, 80 and 100). The summary of 24-h and 48-h acute toxicity of water samples of 4 stations in Nif Brook presented in Figure 2. In this study the 95% confidence limits overlap and dose-response relationship was defined.

Water samples in station 1 of the Nif Brook were found to be more toxic in the test. The number of Immobilize Daphnids rates was increasing parallel to increasing water dilutions in all stations. In the mean time, any death was found in the control group. At Series of water samples containing 10 % of all stations percentage of immobilize daphnids observed as 12, 5, 12 and 11 for station 1, 2, 3 and 4 at 48-h (Table 1). Toxicity of water samples of all Stations to daphnia as LC50 after 48-h exposure ranged from 6.8 % to 27.18 % in water. The 48-h acute test results, average LC50 for *D.magna* was calculated as 6.8 % for station 1, 27.178 % for station 2, 9.1 % for station 3 and 12.67 % for station 4 respectively.

It can be concluded that comparing LC50 levels of the elutriate dilutions the toxicity order is Station 2< Station 4< Station 3< Station 1 (Table 2).

48-h acute tests, the effects of water samples taken, the comparison was made between stations were significantly different ($p<0.05$). Daphnids were exposed to the following concentrations of sediment for 48-h: control, 2.5, 5, 10 and 15 µg/L.

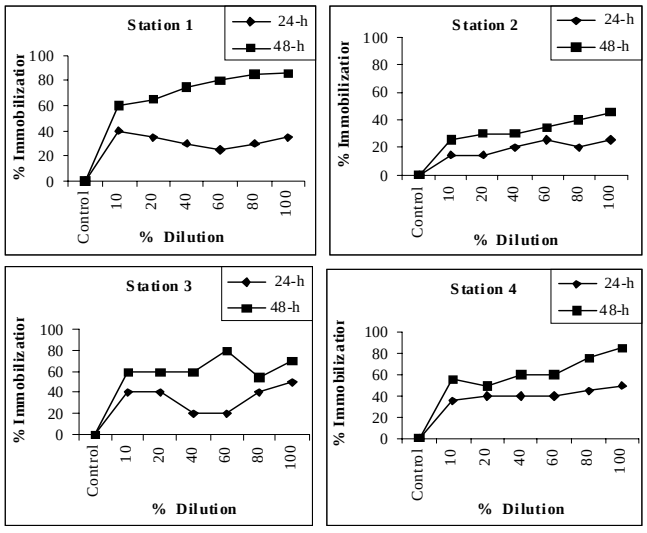

Figure 2. Immobilization of *D.magna* exposed to the diluted elutriates of the waters from 4 stations in Nif Brook.

The summary of 24-h and 48-h acute toxicity of sediment samples of 4 stations in Nif Brook presented in Figure 3. In this study the 95% confidence limits overlap and dose-response relationship was defined. Sediment samples in station 1 of the Nif Brook were found to be more toxic in the test.The number of Immobilize Daphnids rates increased parallel to increasing sediment samples in all stations.

Table 1. Acute test results of water from 4 stations from Nif Brook. *Statistically significant decrease comparing to controls.

% Dilution	Total Number of Individual	Number of Immobolization	% Number of observed Immobilization
Control	20	0	0
Positive Control	20	20	100
Station 1			
10	20	*12	60
20	20	*13	65
40	20	*15	75
60	20	*16	80
80	20	*17	85
100	20	*18	86
Station 2			
10	20	*5	25
20	20	*6	30
40	20	*6	30
60	20	*7	35
80	20	*8	40
100	20	*9	45
Station 3			
10	20	*12	60
20	20	*12	60
40	20	*12	60
60	20	*16	80
80	20	*11	55
100	20	*14	70
Station 4			
10	20	*11	55
20	20	*10	50
40	20	*12	60
60	20	*12	60
80	20	*15	75
100	20	*17	85

* $p<0.05$

In the mean time, no death was found in the control group. At Series of sediment samples containing 2.5 µg/L of all stations percentage of immobilize daphnids observed as 2.5, 5, 20 and 20 for station 1, 2, 3 and 4 at 48-h. At highest test medium contain 15 µl/L immobilization reached approximately % 70 in stations (Table 2). Toxicity of sediment samples of all Stations to daphnia as LC50 after 48-h exposure ranged from 6.826 to 38.038 µg/L in water.

The 48-h acute test results, average LC50 for *D.magna* was calculated as 6.8 % for station 1, 38.038 % for station 2, 9.774 % for station 3 and 21.198 µg/L for station 4 respectively. It can be concluded that comparing LC50 levels of the elutriate dilutions the toxicity order is Station 2< Station 4< Station 3< Station 1. 48-h acute tests, the effects of sediment samples taken, the comparison was made between stations were statistically significant differences ($p<0.05$). The effect of water and sediment of 4 stations on daphnids survival and reproduction was investigated in 7-day chronic studies. LC50 values, for Nif Stream Water Samples; 0.68 µl /L of station, 0.27 µl /L of station 2, 0.9 µl /L of station 3, 4 station is 12.6 µl /L water samples have been found for Nif Stream.

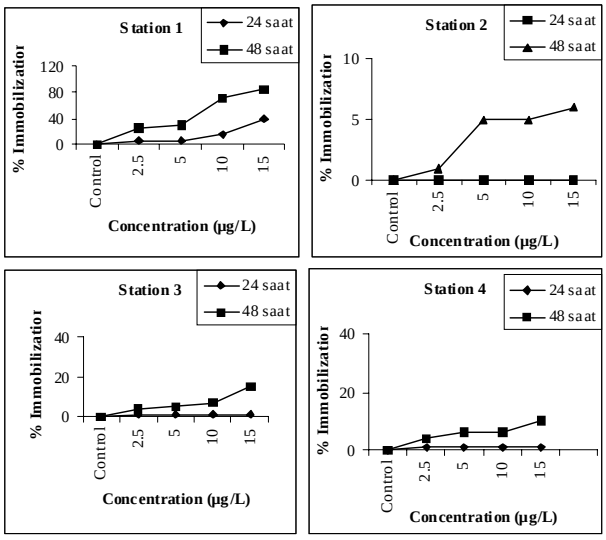

Figure 3. Immobilization of *D.magna* exposed to the sediment samples of the sampling stations.

Table 2. Acute test results of sediments from 4 stations from Nif Brook. *Statistically significant decrease comparing to controls.

Sediment Samples (µg/L)	Total Number of Individual	Number of Immobolization	% Number of observed Immobolization
Control	20	0	0
Positive Control	20	20	100
Station 1			
2.5	20	*5	*25
5	20	*6	*30
10	20	*14	*70
15	20	*17	*85
Station 2			
2.5	20	*1	*5
5	20	*5	*25
10	20	*5	*25
15	20	*6	*30
Station 3			
2.5	20	*4	*20
5	20	*5	*25
10	20	*7	*35
15	20	*15	*75
Station 4			
2.5	20	*4	*20
5	20	*6	*30
10	20	*6	*30
15	20	*10	*50

*p<0.05

Acute LC50 values were calculated as a result of the test water and sediment samples obtained in chronic tests, LC50 values 1/10 were used for calculation. Chronic lethal effect was not observed in the test concentrations for Stations 1,2,3 and 4 sediment samples consisted of, 0.68 mg / L, 3.8 mg / L, 0.98 mg / L and 2.12 mg / L concentrations of sediment samples respectively.

The results showed that the water of all stations produced low survival. The water of station 1, 3 and 4 produced positive effects (hormeosis) on the number of brood productions except for station 2 from Nif Brook.

The hormesis effect was observed on the number of young produced with 43% of station 1, 47% of station 3 and 58% station 4. Adult survival was %60, %75, %95 and %80 in the sediment samples (Table 3). The results showed that the sediment samples of 4 stations in Nif Brook produced low survival. All of the sediment samples from all stations produced negative effects on the number of neonates and total number of molts per individual. The comparison was performed between samples and control group were found statistically significant differences ($p<0.05$).

Discussion and Conclusion

Two test design were used in this study. Lethal Effects (lethality) of water and sediment were determined with acute tests and, sub-lethal effects (survival, reproduction, immobility) were investigated chronic tests. Daphnia magna is one of the most important fresh water species employed in ecotoxicity testing for effluents, water and sediment samples. In previous researches *D.magna* were exposed to whole sediment, filtered or unfiltered sediment elutriates or sediment pore waters in acute sediment toxicity test (Schuytema et al. 1984, Hall et al. 1986, Ristola et al. 1996). Soucek et al (2000) had reported the 48-h LC50 values of pore water and elutriates of sediment samples as 35% and 65% respectively. This results show that the elutriate of the sediment reflects the toxicity of sediments better than pore water. Bridges et. al. (1996) had investigated the toxic effects of the sediments of Great Lakes and reported that the undiluted elutriates were decrease the survival rate up to 50 %. Dave and Nilsson (1999) determined toxic effects of Kattegat and Skagerrak sediments in Baltic Sea using two species D.magna and Nitocra spinipe and found similar results. Ingersol (1992) found that the acute toxicity of irrigation drain waters to *D.magna*. Contrary, Ristola et. al. (1996) had recorded that sediment elutriate or pore water tests did not exhibit toxicity to *D.magna* in Lake Ladoga sediments. However, This is in marked contrast to the results of an extensive study by Ankley et. al. (1991), who examined the effects of pore water, elutriate and solid-phase fractions from 29 different sediments on four different species of aquatic organisms. The authors found that pore water bioassays were the most toxic component of the three phases and that, although elutriates were poor predictors of toxicity from contaminated sediment, pore water tests correlated well with bulk sediment toxicity. However, they point out that the solid-phase experiments were, in fact, diluted to a greater extent than pore water tests. The results in the current study support the observed toxicity noted in the freeze-dried sediment.

Nif stream water and sediment samples were taken on the 4 station in the toxicity tests carried out in various concentrations. Test results showed that the water and sediment samples of all stations of brook has stopped the

Table 3. Sublethal effects waters and sediments from 4 stations from Nif Brook. *Statistically significant decrease comparing to controls. ** Statistically significant increase (hormeosis) comparing to controls.

Parameter / Station	Survival %	Total number of molts per individual	Total numbers of neonates
Control	100	5.72	40
Water			
1	*70	6.14	43
2	*75	*2.29	*16
3	90	**6.71	**47
4	95	**8.79	**58
Sediment			
1	*60	5.42	38
2	*75	*2.14	*15
3	95	*3.86	*27
4	80	5.14	36

*$p<0.05$

survival of adult Daphnids at the end of 48-h. Nif Brook water samples taken from station 1, which is output of OSB, was produced negative effects on growth of D.magna. Earlier in 2002, Nif stream sediment samples taken from the same stations, all stations of the mutagenicity study showed weak mutagenic and toxic effects (Boyacioglu 2002). In 2009, Arslan and his colleagues received the same stations in the sediment and water samples made with sea urchin (Paracentrotus lividus) embryos and derivation embryotoxicity study is stopped as a result of toxic Nif stream and caused developmental abnormalities have been reported (Arslan et al., 2009b). The results of our study are consistent with the results of the above studies. On the other hand the numbers of eggs produced by D.magna were increased in sub lethal test dilutions of water samples from stations 1, 3 and 4 except station 2 of Nif Brook. This situation were reported in many research (Stebbing 1881, Pagano et. al. 1986) and defined as "hormesis" meaning "stimulated reproduction under the unfavorable conditions". The effect of unfavorable conditions as increased number of egg but the survival of adults was decreased.

Results of this study showed that, screening the toxicity of polluted waters and sediments by using D.magna gives better and more meaningful results than an quantitative analyze of toxic matters. Additionally, it is noted that this method is economic, easy to apply and repeatable. In this matter, not only the environmentally polluted waters but also the toxicity of industrial discharge water could be able to be screened by sediment toxicity test using D.magna.

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