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An Assessment of Sediment Quality at the Streams Flowing into Izmir Bay, Aegean Sea, Turkey

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Abstract: Due to municipal, industrial and nonpoint source waste discharges to streams and rivers and tendency of many chemicals to become associated with sediments. Sediments are particularly problematic near densely populated and industrialised urban areas, such as the Izmir metropolitan area in west part of Turkey. The aim of this study was to determine the potential adverse effects of sediments from five streams flowing into inner part of Izmir Bay by using sea urchin, *Paracentrotus lividus* embryotoxicity test and if there was any correlation between toxicity and chemical data. Toxicity tests indicated that with the exception of the smallest concentration of only one stream sediment samples (0.6 mg wet wt. mL^{-1}) all sediment samples resulted in significant increases in the frequencies of developmental defects on *P. lividus* embryos. Analytical data showed that the sediments from five streams had chemical characteristics similar to sediments defined by other authors as polluted sediments. An important point to note was the excellent correlation between total organic carbon content of stream sediments and sea urchin *P. lividus* embryotoxicity data, but not with metal content.

Key words: Sea urchin, embriotoxicity, sediment, metals, organic carbon, Izmir Bay

INTRODUCTION

The sediments in aquatic systems are a sink to variety of contaminants and can be the cause of stress to the biota. Various biological tests have been used to assess the biological effects of contaminated sediments (Hoke *et al*., 1993; Matthiessen *et al*., 1998; Geffard *et al*., 2004). Among these bioassays, using sea urchin gametes and embryos appear to have numerous advantages for aquatic toxicity testing their sensitivity to a wide variety of environmental contaminants (De Nicola *et al*., 2004; Gulliani *et al*., 2002; Pagano *et al*., 1996; Trieff *et al*., 1995). In addition, sea urchins have gained widespread acceptance as a test species for testing effluents and other various types of aqueous extracts (Saotome and Hayashi, 2003; Meriç *et al*., 2005). Furthermore, several investigations have evaluated the high sensitivity of sea urchin to contaminated fresh water sediment (river, stream, etc.) marine sediment and soil (Pagano *et al*., 1993; Pagano *et al*., 2002; Beiras, 2002).

Izmir Bay is located in the western part of Turkey and polluted by several sources which are mainly untreated or partially treated domestic and industrial wastes, urban and agricultural run-offs and discharges from ships as well as from commercial harbours. The streams collect most of the industrial, agricultural and domestic effluents and discharge them into Izmir Bay. As these streams are one of the main pollution carriers to Izmir Bay, the researches on the toxic effects of them have remarkable importance for the following clean-up efforts for the bay.

The aims of this research were as follows; (1) to evaluate the toxicity of sediments from five streams flowing into the inner part of Izmir Bay, Aegean Sea, Turkey by using sea urchin, *Paracentrotus lividus* embryotoxicity test; 2) and to determine if there is correlation between toxicity and chemical data.

MATERIALS AND METHODS

Sediment samples were collected from the Meles Stream, Arap Stream, Manda Stream, Bornova Stream and Bostanli Stream which are flowing into the inner part of Izmir Bay on Aegean Sea, Turkey as depicted in Fig. 1. Sediment sampling was performed on 6 November 2000 and 2 February and 14 May 2001. The samples were collected with Van-Veen grab from the top 15 cm of the sediment layers defined as the surface sediments. The sediment samples were removed from the sampler and the outer parts separated to prevent contamination from metallic body of the sampler. Teflon spatula was used for

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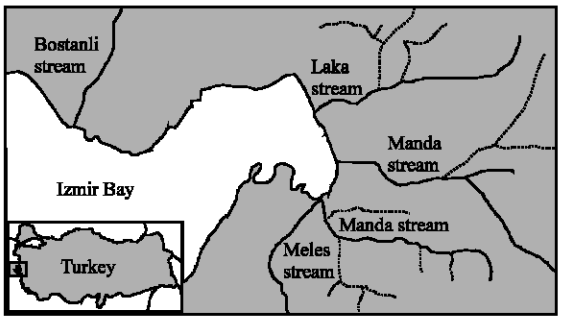

Fig. 1: Location of the sampling points

the same purpose. The samples were placed in which are pre-cleaned with diluted nitric acid and rinsed with distilled water plastic bags and kept in an icebox until arriving to the laboratory, where they were maintained at approximately 4°C until processing and analysis.

For inorganic analyses samples were dried in an oven at 60°C for approximately 3-4 days. Dried samples were homogenized and sieved to fine powder. Samples were solubilized using wet-digestion system. Approximately 0.5 g dried and homogenized sediment was weighed and placed into the acid-washed containers to which a 5 mL mixture of perchloric acid and nitric acid (1:5 V: V) were added. The containers were connected to water condenser to prevent the loss out of acid fume. The containers were placed on the water bath heated 140°C for 12 h keeping continuous flow of cool water through the condenser. The digested samples were transferred into a 50 mL polyethylene flask, diluted to 50 mL with bidistilled water and the flask transferred into pre-cleaned with acid wash polyethylene bottles for storage. Special attention was paid for the reagent to be supra pure grade for digestion process. Triplicate samples were prepared from each sediment sample and the same procedures were applied for the blank samples (APHA/AWWA/WPCH,1989). The samples were analysed by flame AAS (Varian Spectra-300 plus) to determine chromium, cobalt, nickel, copper, arsenic, cadmium, ferrous, zinc, manganese and lead concentrations. The certified reference materials were used to check the accuracy and reliability of the method. The results were given in mg kg^{-1} dry weight.

Organic carbon in sediment samples was analysed using the methods previously reported (Gaudette and Flight, 1974). To analyse organic carbon a 0.2 to 0.5 g air dried and sieved sediment sample placed in a 500 mL Erlenmeyer flask. Exactly 10 mL of 1 N $K_2Cr_2O_7$ solution was added to the sediment and the two were mixed by swirling the flask. Twenty milliliter of concentrated H_2SO_4 were added and mixed by gentle rotation of the flask for about one minute. This was done carefully to insure complete mixing of the reagents with the sediment, while avoiding throwing the sediment onto the sides of the flask out of contact with the reagents. The mixture was allowed to stand for 30 min. A standardization blank without sediment was run with each new of samples. After 30 min, the solution was diluted to 200 mL volume with distilled water and 10 mL 85% H_3PO_4, 0.2 g NaF and 15 drops of diphenylamine indicator were added to the sample flask. The solution was back titrated with 0.5 N ferrous ammonium sulphate solution. The colour would progress from an opaque green brown, to green upon the addition of approximately 10 mL of ferrous solution. The colour would continue to shift upon titration to a black grey; at this point the edition of 10-20 drops of ferrous solution would shift the colour to a brilliant green giving a one-drop end point.

Adult sea urchins *Paracentrotus lividus* (Lamarck, 1816) were collected from the unpolluted area of Izmir Bay. Gametes were harvested and embryos were reared as previously described (Pagano *et al*., 1986). Controls throughout experiments were conducted as untreated negative controls (filtered natural sea water, FSW, collected from Seferihisar (unpolluted area of Eagean Sea) and positive controls (exposure to 2.5 10^{-4} M $CdCl_2$). Each experiment was run in triplicates, using six-well plates (Nunc, Denmark). In each replicate, eggs from one female were fertilised by sperm pooled from two males. Ten minutes after *in vitro* fertilisation, a 1 mL aliquot of zygote suspension of approximately 300 embryos/mL was added gently to sediment (0.6, 2, 6 mg mL^{-1}, wet settled sediment) contaminated seawater. The embryos were reared up to the pluteus larval stage (72 h) at 18±1°C in 10 mL filtered Natural Sea Water (FSW) (approx. 30 embryos mL^{-1}). After each test, 100 embryo or larvae were scored as frequency of developmental defects on living 72 h-old larvae immobilized in 10^{-4} M chromium sulphate. The following outcomes in embryogenesis were evaluated: i. Retarded (R) plutei [≤1/2 size vs. Normal (N) plutei]; ii. Pathologic (P1), malformed plutei; iii. Pathologic embryos (P2), unable to differentiate up to the pluteus larval stage and iv. Dead (D) embryos/larvae (Pagano *et al*., 1986).

Data were currently analysed for statistical significance using the one-way ANOVA test. Significance level of statistical analyses was set at $\alpha = 0.05$. Linear regressions between larval malformations in sea urchin and metal and organic carbon contents of stream sediments were determined using Excel software.

RESULTS AND DISCUSSION

The effects of five stream sediment samples on embryonic development of sea urchin were examined in

three experiments in triplicate. As shown in Table 1 with the exception of the smallest concentration of Meles Stream sediment samples (0.6 mg dry wt. mL^{-1}) all sediment samples resulted in significant increases in the frequencies of developmental defects on *P. lividus* embryos. The highest developmental toxicity was exerted by Bostanli Stream and Manda Stream sediments on *P. lividus* embryos, respectively. The embryotoxicity of sediment samples of Meles Stream, Arap Stream and Laka Stream were observed to have larval malformations (P1) at pluteus stage. However, the significant amount of embryos were unable to attain the pluteus larval stage (e.g., abnormal blastulae) and die at embryonic or larval stage when exposed to Bostanli Stream and Manda Stream sediments.

Heavy metal concentrations in sediments from the streams which flowing into inner part of Izmir Bay on Aegean Sea (Turkey) varied according to the sampling station and the difference was statistically significant (all of them $p<0.05$). The maximum and minimum amounts of heavy metal concentrations in the stations were, respectively Co: 4.92-3.4; Cu: 135.7-24.2; Mn: 106.0-53.2; Cr: 128.4-8.78; Pb: 89.6-23.4; Fe: 2335.0-1497.0; Cd: 1.39-0.47; Ni: 38.42-9.0; Zn: 953.3-572.5 mg kg^{-1} dry weight (Table 2).

Heavy metal concentrations exhibited variations depending upon the different compositions of effluents discharged in the streams. One of the way at determining the level of the metals contaminated in the streams sediment is to compare the results obtained with the average shale standards (Turekian and Wadepohl, 1961). As Facetti *et al.* (1998) stated that the shale standard, based on a large number of samples and assumed to be uncontaminated is considered to be the best medium for comparison with recent basin sediments. Some of the heavy metals studied in this research appeared to have average concentrations higher than the shale standards (Table 2). Zn, Cd, Fe and Pb in all sites, Cu in Arap Stream and Laka Stream, Cr in Laka Stream exceeded the shale standard. It can be concluded that the heavy metal concentrations studied were originated from anthropogenic sources as effluents originated from many types of industrial, domestic, agricultural wastes and the airborne particles. On the other hand, the sediments from five streams running to Izmir Bay have chemical characteristics similar to sediments defined by some other authors as polluted sediments (Dauvalter and Rognerud, 2001; Akcay *et al.*, 2003).

Percentage of total organic carbon in the sediments ranged from 1.5 to 13.1% (wt) showing remarkable differences according to the effluents types which were discharged to the streams. Statistical data of the recent study suggest that there was strong correlation between organic carbon contents and toxicity outcomes of sediment samples (Table 3) as seen in several studies (Hoke *et al.*, 1993; Geffard *et al.*, 2002). Although the

Table 1: Developmental toxicity as induced by sediment samples from five streams flowing into Izmir Bay. Three triplicate experiments on *P. lividus* embryos (X±SE)

Sediment Concentrations (mg mL^{-1})	P1	P2	D
Blank	1.3±0.3	0.0±0.0	0.0±0.0
Meles Stream			
0.6	4.8±2.3	0.1±0.1	0.0±0.0
2	13.0±3.4*	0.1±0.1	0.1±0.1
6	35.1±5.1*	0.7±0.7	0.0±0.0
Arap Stream			
0.6	9.0±2.1*	0.0±0.0	0.0±0.0
2	21.0±5.4*	0.4±0.2**	0.0±0.0
6	81.7±4.3*	2.1±1.4*	0.0±0.0
Laka Stream			
0.6	11.0±4.2*	0.0±0.0	0.0±0.0
2	38.1±6.3*	0.0±0.0	0.0±0.0
6	89.2±3.2*	2.3±1.0*	0.0±0.0
Manda Stream			
0.6	36.4±6.7*	0.4±0.2**	0.2±0.2***
2	77.1±4.9*	3.8±2.1*	1.3±1.0***
6	17.1±9.0	59.8±10.5*	23.1±1.3*
Bostanli Stream			
0.6	48.3±7.7*	1.9±1.3*	0.0±0.0
2	51.0±13.7*	44.2±14.4*	2.11±1.9*
6	0.0±0.0***	42.9±1 1.8*	57.1±12.0*

*p<0.001, **p<0.005,***p<0.05

Table 2: Heavy metal analyses and organic carbon contents of the sediment samples collected from the streams flowing into Izmir Bay (X±SE)

Metal (mg/kg)	*Shale standard	Meles stream	Arap stream	Laka stream	Manda stream	Bostanli stream	p-value
Co	19	4.9±0.8	4.6±0.5	4.3±0.0	3.4±0.2	3.4±0.0	p<0.005
Cu	45	24.2±4.8	135.7±29.0	66.2±16.4	35.5±6.6	39.0±1.8	p<0.00001
Mn	850	106.0±18.0	90.8±14.3	65.8±5.3	53.2±4.4	61.2±2.7	p<0.00001
Cr	90	18.4±1.2	40.6±3.5	128.0±24.0	25.8±12.3	8.8±0.2	p<0.00001
Pb	20	28.0±4.6	37.4±3.0	89.6±16.4	23.4±5.7	28.6±3.4	p<0.00001
Fe	200	2664±413	2048±119	2316±23	1497±125	2335±32	p<0.0005
Cd	0.3	0.58±0.1	1.39±0.2	0.8±0.2	0.6±0.2	0.5±0.1	p<0.00001
Ni	68	24.7±3.2	38.42±4.3	34.7±8.4	11.3±0.8	9.0±0.5	p<0.00001
Zn	95	900.6±134.0	572.5±97.0	953.3±62.7	746.7±24.2	863.3±43.6	p<0.005
Organic Carbon%		1.5±0.4	9.9±5.2	8.0±0.3	11.0±3.6	13.1±0.9	

*(Turekian and Wadepohl, 1961)

Table 3: Equation of the relationship between organic carbon contents and toxicity outcomes of all stream sediments

Sediment concentrations in FSW (mg mL^{-1})	Equation	n	r^2
0.6	Y = 11.876x-13.186	5	0.8781
2	Y = 13.819x-13.869	5	0.7578
6	Y = 20.011x-1.52	5	0.8234

sediment concentrations in the experiment were increased, the correlation did not change much. Besides, the toxicity result did not show a significant correlation between the effects on embryonic development of sea urchin and sediment metal concentrations. However some of the streams exceeded shale standards in some of the metals. The lack of significant correlation between the toxicity data and metal contents in stream sediments maybe attributed to un-bioavalibility of the metals to be uptaken by embryos of sea urchin. It clearly seems that bio-availability of these metals may have been decreased possible changes in their chemical specifications when diluted with sea water and causing a decrease in their embryo toxic effect.

CONCLUSION

The toxicity outcomes of stream sediment displayed a significant dose-related increase of larval malformations and differentiation arrest in *P. lividus* at all data sets. In conclusion, the result here suggest that sea urchin embryos are a sensitive tools for evaluating biological quality of contaminated sediment with an excellent agreement with previous studies (Pagano *et al*., 1993; Pagano *et al*., 2002; Beiras, 2002).

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