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Kraft Pulp Mill Effluent and Sediment Can Retard Development and Lyse Sea Urchin Eggs

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The biological effects of discharges derived from kraft pulp and paper mill on aquatic organisms have been well documented by FUJIYA (1962) and WALDEN (1976). These effects included acute and sublethal toxicity on fishes and bivalves, and incipient changes in the environmental threshold levels. The wastes from pulp and paper mills are a complex mixture of mainly organic and inorganic moieties (LEACH et al. 1975, 1977; THAKORE 1977; SEIM et al. 1977). Hence, a variety of natural or chlorinated resin acids, unsaturated fatty acids, diterpene alcohols, natural and chlorinated phenolics and juvabiones have been implicated as the most probable toxic elements towards aquatic organisms (DAVIS 1973; DAVIS et al. 1973; WALDEN et al. 1975; ROGERS et al. 1975; STOCKNER et al. 1976).

We previously have identified 28 different organic compounds including resin acids, phenol derivatives and aromatic hydrocarbons by gas chromatography and mass spectrography analysis from the sediment of the estuary of Shinguu river, Wakayama Prefecture, Japan (KINAE et al. 1981). A fairly large pulp and paper mill situated along the river discharges enormous amounts of organic materials into the water.

The present study analyses ether extracts obtained from the effluent and sediment of kraft pulp mill with regard to their effects on the sea urchin (Anthocidaris crassispina) eggs. This procedure, introduced by KOBAYASHI (1971), is a convenient means to monitor the toxic marine pollutants.

MATERIALS AND METHODS

Sampling of Effluent and Sediment

The samples of effluent and sediment were collected from the drain-pipe and the bay neighboring a kraft pulp mill on the Shinguu river in March 1979. These samples were stored at $4^{0}C$ in tightly sealed glass bottles.

Chemical Analysis of the Samples

The chemical and physical properties of the effluent and sediment were examined according to the Standard Method of Analysis for Hygenic Chemist (1973). The amount of sodium ligninsulfonate was determined by the method of FELICETTA et al. (1963).

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Extraction and Separation of Organic Compounds

Two liters of the effluent were saturated with solid NaCl and acidified with 5% HCl and then extracted with 400 ml of distilled diethylether (ether). The extraction was repeated twice and combined ether extracts were treated according to the method of BRAUS et al. (1952) to separate it into neutral, acidic, basic, amphoteric, and phenolic fractions. The ether extracts were resuspended in acetone after evaporation of the ether.

The sediment (1 Kg wet weight) was mixed with 2 L of distilled water, stirred for 1 hour at room temperature, and filtered through a 40 µM glass filter. The residue was treated with an equal volume of methanol and again filtered. The two filtrates were combined, evaporated at 40^0C to approximately 50 ml, saturated solid NaCl, and extracted 2 times with 30 ml of ether. The ether extracts were then treated similar to the effluent samples to obtain five fractions.

Effects of Sediment and Effluent Extracts on Sea Urchin Eggs

Experiments were conducted according to the method of KOBAYASHI (1971). Spermatozoa and eggs were removed from the male and female sea urchins with an injection of 0.5 M KCl. The sperm suspension (0.1 ml) were added to a 10 ml egg suspension (approximately 1000 spermatozoa and 3000 eggs/10 ml) in sea water containing a 0.1 ml acetone solution of the extracts at the various concentrations to be tested (Results). The reaction mixture was incubated at room temperature after gentle agitation. After 3 min, 90 min, 12 hr, and 24 hr of incubation, samples were fixed with 5% formalin, and microscopically examined for the degree of cell division, blastulation, and gastrulation of fertilized eggs (Figure 1).

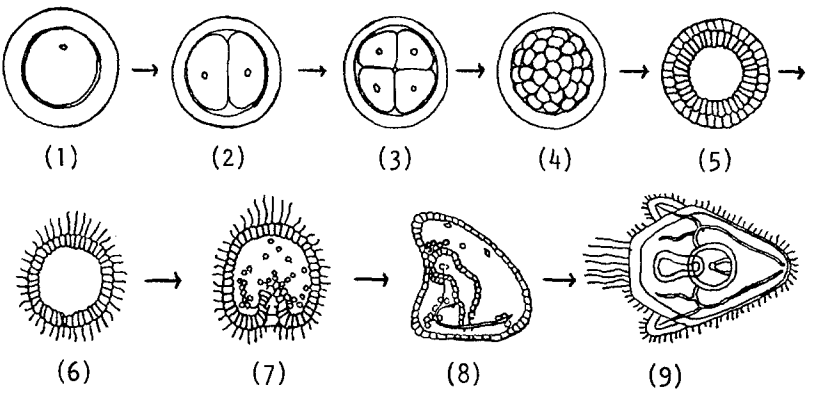

Figure 1. Schematic drawing for the development of sea urchin (Anthocidaris crassispina) eggs at 25^0C
(1): The stage of fertilization (3 min after the fertilization)
(2)-(3): The stage of cell division (60-90 min after)
(4)-(6): The stage of blastulation (3-12 hr after)
(7)-(8): The stage of gastrulation (14-24 hr after)
(9): The stage of pluteus (30 hr after)

The mutagenic and carcinogenic agents, 2-(2-furyl)-3-(5-nitro-2-furyl)acrylamide (AF-2) and benzo(a)pyrene (B(a)p), served as positive controls. Also, as positive toxic controls, size chemicals, wet strengthen chemical, slime controllers, agglutants, and surfactant, all of which are used in pulp and paper industries, were similarly examined for their effect on the sea urchin eggs. The negative control consisted of sea water containing 1% acetone. All experiments were conducted at least in duplicate.

RESULTS

The chemical properties of the effluent and the sediment samples used in these experiments are shown in Table I. Although the effluent sample was weakly acidic, the sediment was weakly alkaline and exhibited a relative high lead and arsenic content.

The effluent and sediment samples were systematically extracted with ether into five fractions according to the method of BRAUS et al. (1952). The color and yield of the ether fractions separated from these samples are shown in Table II. Among these five fractions, the acidic fraction of the effluent sample was the most abundant (5.0 mg/L), while the neutral fraction was the highest (136.9 mg/Kg) from the sediment sample.

The effects of the ether extracts from the effluent and sediment samples on sea urchin eggs are shown in Table III and IV, respectively. Among five different fractions from the effluent extracts, the basic fraction was the most toxic and decreased the fertilization of the eggs to 35% at 10 ppm concentration. All five fractions retarded the development of sea urchin eggs at 1 or 10 ppm concentration and showed cytolysis at 100 ppm concentration.

TABLE I

Chemical and physical characteristics of effluent and sediment samples

Items	Samples	
	Effluent	Sediment
Color	Yellow-brown	Black
Odor	Pulp-like smell	Rotten-egg smell
pH	6.72	7.40
COD (ppm)	95.5	-
Loss on drying (%)	-	61.9
Cl ion (ppm)	224	4.91×10^3
Total S (ppm)	0.19	0.69
Lignin (ppm)	32.5	3.20
Pb (ppm)	ND*	16.4
Cd (ppm)	ND	0.44
Zn (ppm)	0.12	54.0
Hg (ppm)	1.50×10^{-4}	0.26
As (ppm)	ND	3.55

* ND: Not detected

TABLE II

Color and yield of ether extracts obtained from effluent and sediment

Samples	Ether fractions	Color	Yield[1]
Effluent:	Neutral	Yellow	2.1
	Acidic	Yellow	5.0
	Basic	Yellow	1.1
	Amphoteric	Colorless	0.3
	Phenolic	Brown	2.8
Sediment:	Neutral	Red-brown	136.9
	Acidic	Yellow-green	6.7
	Basic	Yellow	2.2
	Amphoteric	Colorless	5.2
	Phenolic	Yellow	9.8

[1]Numbers indicate mg/L for effluent and mg/Kg for sediment, respectively.

TABLE III

The effects of ether extracts from the effluent on sea urchin eggs

Ether fractions	Conc (ppm)	Development ratio (%)					Results[2]
		Fertilization	Cell division		Blastulation[1]	Gastrulation	
			2 Cells	4 Cells			
Neutral:	1	100	9	73	(6)	0	R
	10	100	35	38	(6)	0	R
	100	17	29	4	(4)	0	L
Acidic:	1	100	7	76	(6)	0	R
	10	50	13	57	(6)	0	R
	100	33	0	0	-	0	L
Basic:	1	100	0	70	(5)	0	R
	10	35	30	0	(5)	0	R
	100	25	0	0	-	0	L
Amphoteric:	1	90	8	70	(6)	0	R
	10	60	10	40	(6)	0	R
	100	40	25	5	(5)	0	R
Phenolic:	1	100	55	15	(6)	0	R
	10	95	65	15	(6)	0	R
	100	60	0	0	-	0	L
B(a)P	1	100	70	15	(6)	0	L
Control (1% Acetone)		100	20	80	(6)	100	N

Both eggs and spermatozoa of Anthocidaris crassispina were added to the sea water containing 1% acetone solution of sample and kept at 25°C.

[1]Numbers in parentheses indicate the stage of fertilized egg development shown in Figure 1.

[2]Sea urchin eggs were monitored for normal (N) appearance, retardation (R) of growth, and cellular lysis (L).

In the sediment samples, the acidic fraction was the most toxic and decreases the fertilization of the eggs by 25% at a 10 ppm concentration. All five fractions from sediment samples inhibited the development of sea urchin eggs at concentrations of 10 and 100 ppm as was the case with the effluent extracts.

The toxic effects of manufacturing materials which have been commonly used in pulp and paper industries are shown in Table V. Slime controller formulations A and B contained bromine and nitro groups respectively, were the most toxic among the materials tested producing cytolysis of the eggs at 1 ppm concentration.

DISCUSSION

It has been known that the discharge from kraft pulp and paper mills contains many substances toxic to fish and planktons.

TABLE IV

The effects of ether extracts from sediment on sea urchin eggs

Ether fractions	Conc (ppm)	Development ratio (%)					Results[2]
		Fertilization	Cell division		Blastulation[1]	Gastrulation	
			2 Cells	4 Cells			
Neutral:	1	85	81	14	(5)	0	R
	10	56	19	39	(5)	0	R
	100	0	0	0	-	0	L
Acidic:	1	93	23	46	(4)	0	R
	10	25	28	25	(4)	0	R
	100	0	0	0	-	0	L
Basic:	1	96	4	76	(6)	0	R
	10	63	12	52	(6)	0	R
	100	17	5	0	-	0	L
Amphoteric:	1	98	5	75	(6)	0	R
	10	64	29	67	(6)	0	R
	100	30	25	0	-	-	L
Phenolic:	1	86	4	72	(6)	100	N
	10	50	27	0	(4)	0	R
	100	31	0	0	-	0	L
AF-2	1	90	60	20	(6)	30	L
	10	86	55	14	(5)	10	L
	100	83	55	4	(4)	0	L
Control (1% Acetone)		100	20	80	(6)	100	N

The experiment was done in the same way as shown in the margin of Table III.

[1]Numbers in parentheses indicate the stage of fertilized egg development shown in Figure 1.

[2]Sea urchin eggs were monitored for normal (N) appearance, retardation (R) of growth, and cellular lysis (L).

TABLE V

The effects of pulp and paper manufacturing materials on sea urchin eggs

Manufacturing materials	Formulation[1]	Conc (ppm)	Development ratio (%)					Results[3]
			Fertilization	Cell division		Blastulation[2]	Gastrulation	
				2 Cells	4 Cells			
Size chemical	A:	1	100	80	10	-	0	L
		10	100	75	4	-	0	L
		100	80	30	0	-	0	L
	B:	1	100	100	0	(4)	0	L
		10	60	5	0	-	0	L
		100	25	0	0	-	0	L
Wet strengthen chemical:		1	100	65	15	(4)	0	R
		10	100	60	10	(4)	0	L
		100	100	40	0	-	0	L
Slime controller	A:	0.01	80	30	50	(6)	100	N
		0.1	80	0	0	(4)	0	R
		1	50	0	0	-	0	L
	B:	0.01	100	30	60	(6)	90	N
		0.1	97	30	60	-	0	R
		1	85	0	0	-	0	L
Agglutant	A:	100	100	60	0	(4)	0	R
	B:	100	100	75	10	(4)	0	R
	C:	100	100	55	5	(4)	0	L
Surfactant:		1	100	40	5	(6)	30	R
		10	80	0	0	-	-	R
		100	0	0	0	-	0	L
Control (1% Acetone)			100	20	80	(6)	100	N

The experiment was done in the same way as shown in the margin of Table III.

[1]Manufacturing materials are of varied unpublished chemical formulation.

[2]Numbers in parentheses indicate the stage of fertilized egg development shown in Figure 1.

[3]Sea urchin eggs were monitored for normal (N) appearance, retardation (R) of growth, and cellular lysis (L).

Several bioassays have been developed to determine the toxicity of the discharge. The method of KOBAYASHI (1971) using sea urchin eggs displays such advantages as simplicity and high sensitivity for toxic marine pollutants.

From the results of our present study, the minimum concentration affecting the development of sea urchin eggs was 1 ppm for the ether extracts from both effluent and sediment samples. Among nine manufacturing materials relating to pulp and paper industries examined, the materials known as slime controller were the most toxic for sea urchin eggs.

It is possible that elements deposited from pulp and paper plants could be responsible for many neoplasms of fish such as melanoma of Nibea mitsukurii (KIMURA 1976). In this regard, we noted that the ether extract of the sediment samples from a bay near the kraft pulp mill on Shinguu river contained a relatively large number of organic compounds of potential toxicity to marine life (KINAE et al. 1981). They included 3,4,5,6-tetrachloroguaiacol, 2,4,6-trichlorophenol, 9,10-epoxystearic acid and hydroabietic acid which damaged the DNA of Bacillus subtilis. Also present were fluoranthene and pyrene produced a reverse mutation of Salmonella typhimurium

Presently the compounds screened for their toxic effects on sea urchin eggs are being evaluated for their potential neoplastic properties on Nibea mitsukurii.

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