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EFFECTS OF PURIFIED AND NON-PURIFIED PAPER PLANT EFFLUENTS ON FERTILIZATION AND DEVELOPMENT OF SEA URCHIN *Paracentrotus lividus* Lam., EGGS

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SUMMARY:
The gametes and embryos of *Paracentrotus lividus* were exposed to purified and non-purified effluents of a paper plant together with tap water. The toxicity of purified and non-purified effluents were resembled. Most severe pathologic cases were evident with the tap water. In general, developmental defects were increased with decreasing salinity at all test solutions.

KEY WORDS: Sea urchin, eggs, paper plant, effluents, effects.

INTRODUCTION

Sea urchins long have stimulated the curiosity of many researchers especially of those intended to study reproduction and development. Embryogenesis in the sea urchin has been intensively studied for more than a century. Few studies aimed to study solely the embryology of echinoids from fertilization through pluteus in the past several decades [1]. Besides these, enormous numbers of experiments were conducted on the biological effects of various agents on germ cells and embryos of sea urchins [2] and references cited there in. The use of sea urchin eggs and embryos as an indicator in marine pollution bioassay was first proposed by Kobayashi in 1971 [3]. Studies concerning the effect of effluents from paper mills are very scarce and to the best of authors knowledge, the effect of tannic acid derived from timber-reservoirs or lumber industry was only pronounced earlier by Kobayashi [4]. This study aims to fulfill this gap via studying the effect of purified and non-purified effluents of a paper mill on various developmental stages of *Paracentrotus lividus* Lam., through pluteus.

MATERIALS & METHODS

A) ORGANISMS: Adult sea urchins were collected from the Institute harbour endemic of the region. Rippen eggs and sperms from mature individuals were obtained by the current KCl-method [5]. Sperms from testes were collected into empty glass beakers to

preserve dry. Mature eggs from several females were then pooled and rinsed twice with filtered sea water. Following this, first the eggs were inseminated to one of the duplicate test solutions 15 minutes prior to the addition of sperms to the other solution. Fertilization was initiated after exposure of eggs (30 minutes) and sperms (15 minutes) to solutions by pouring sperms on eggs. The percentage of the eggs with raised fertilization was checked shortly after in first sampling within 15 minutes. Second sampling is done after 48 hours at the stage of pluteus. All samples were preserved in 10% buffered formalin. In addition, salinity and pH of each test solution were measured separately.

B) TEST SOLUTIONS: Purified and non-purified effluents were collected from a nearby Paper Plant (SEKA) as 24 hour composites at two hour intervals. These were then filtered with GF/B Glass Microfibre filters to remove particles. Natural sea water was collected from the Institute harbour and used as the control and the diluent. Tap water was aerated for 24 hours in order to remove excess chlorine and kept overnight in an open glass jar to stabilize the oxygen level. Test solutions were obtained by dilution of tap water, purified and non-purified effluents with filtered natural sea water.

RESULTS & DISCUSSION

The response to purified and non-purified effluent and to tap water of *Paracentrotus lividus* is summarized in Tables 1, 2 and 3.

Table 1. Effects of purified effluent on fertilization and development of *Paracentrotus lividus*.

	15 min. after	48 hours after insemination				pH	Salinity
Conc.	Fert. eggs %	Morula %	Blast. %	Gastr. %	Plutei %		
Control	100			2	98	7.98	34.50
15%	98			44	56	7.98	29.42
20%	98	4	18	63	12	7.91	27.73
25%	96	3	21	57		7.96	26.03
30%	90		6	8		7.92	24.34
35%	73					7.96	22.65
40%	51					8.01	20.95
45%	10					7.90	19.26
50%	1					8.03	17.57

PURIFIED EFFLUENT: It is observed that the purified effluent is the least effective. Serial dilutions up to 40% (Table 1) have yielded large permanent fertilization membranes at 50% of the eggs within 15 minutes. Over this level the success was below 10% and fertilization membranes were hardly seen under microscope and disappeared almost at all eggs at 50% dilution having a salinity of 17.5 ppt. After 48 hours, 56% of the fertilized eggs reached pluteus and the rest were at gastrula in the 15% effluent concentration. No more pluteus was attained at dilutions 25% and over. Few eggs have succeeded blastulation and gastrulation at 30% dilution. Over this dilution all eggs were irregularly divided, in the multi-cell state due to polyspermy, and almost all were severely deformed at higher concentrations at a salinity of 22.65 ppt and below.

NON-PURIFIED EFFLUENT: Retardation and deformations at various stages of development were more conspicuous in comparison to purified effluent. ~50% of the eggs were able to carry on fertilization in the first 15 minutes at 30% effluent concentration. Further development to pluteus was prohibited at 25% effluent concentration and majority stayed at gastrulae. Formation of the fertilization membrane has influenced much at 35% dilution level and stopped totally over this level (Table 2). Following fertilization, critical level for further development was up to 25% effluent concentration around 26 ppt salinity and below this level deformations started and polyspermy were formed.

Table 2. Effects of non-purified effluent on fertilization and development of *Paracentrotus lividus*

	15 min. after	48 hours after insemination				pH	Salinity
Conc.	Fert. eggs %	Morula %	Blast. %	Gastr. %	Plutei %		
Control	100			2	98	7.96	34.50
15%	100		1	5	94	8.00	29.42
20%	99		5	26	69	8.06	27.73
25%	95	9	21	70		8.18	26.04
30%	52					8.26	24.34
35%	18					8.26	22.65
40%	1					8.27	20.96
45%	1					8.25	19.27
50%	0					8.30	17.57

TAP WATER: Most severe pathologic cases were evident with the tap water when compared with the purified and non-purified effluents. 15 minutes later 100% success was achieved in the formation of clearly visible fertilization membranes in controls and in the lowest concentration of tap water (Table 3). With increasing tap water concentrations, very thin, barely seen fertilization membranes became apparent and the rate of fertilization decreased and almost failed at the 35% concentration. After 48 hours the picture was extremely terrible and even at the lowest concentration of tap water (15%) cytolysis and polyspermy were recorded in almost all eggs while 98% of the eggs have reached pluteus in controls during this time. Such a pathologic case was not expected and is challenging. It may be possible that some other factors would effect the fertilization and development of eggs besides salinity. Among these factors, chlorine and trace elements were regarded to be effective. Experiments conducted on sand dollar *Dendraster excentricus* exposed to both chlorinated and unchlorinated West Point, Seattle, Washington effluent prior to fertilization by Stober et al., (1977, cited in [6]) have shown that fertilization was reduced by 50 percent in 4.4% chlorinated effluents. In addition, Kobayashi (1977) has stressed that trace amounts of heavy metals were effective on developing sea urchin eggs [7].

Table 3. Effects of tap water on fertilization and development of *Paracentrotus lividus*

	15 min. after	48 hours after insemination				pH	Salinity
Conc.	Fert. eggs %	Morula %	Blast. %	Gastr. %	Plutei %		
Control	100			2	98	7.86	34.50
15%	100			2		8.02	29.37
20%	92			1		7.99	27.66
25%	77					8.01	25.95
30%	28					8.01	24.23
35%	1					8.07	22.52
40%	0					8.07	20.81
45%	0					8.07	19.10
50%	0					8.04	17.39

Oshida's [6] experiments on purple sea urchin *Strongylocentrotus purpuratus* have shown that fertilization and normal 48-hour development were reduced at salinity levels lower than 28 ppt. The present results are almost consistent with information given by Oshida et

al., in terms of the lower limit of salinity effective on development. Pagano et al., [8] had also reported highly pathological larvae, reared at 75% salinity.

Regarding the effects of pH, it is pronounced earlier that normal fertilization, cleavages and further embryonic development is achieved in the range from 7.8 to 8.6 [4]. As displayed in tables 1, 2 and 3, the pH of the test solutions ranged between 7.86-8.3 in this study. This indicates that any parameter other than pH is controlling the developmental stages. Considering high degree of similarity in salinity and pH levels at all test solutions, crucial chemical analyses of the tap water which strongly inhibited the development at earlier stages is necessary.

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