Chapter 5

Effect of Effluent Contaminated River Water on the Chromosomes of the Frog *Rana tigerina*

A major threat to the survival of amphibian populations has been attributed to the contamination of water sources with industrial effluents (Naik and Vinod, in press). There are several industries in Gujarat State, releasing the untreated or semi-treated effluent to the nearby rivers. The Mini river is one of such rivers in Baroda, which carries the effluents released from many industries, mainly dye and paint factories. Atomic spectra analysis showed a high amount of heavy metal, cobalt in this river water.

There are some reports regarding the effects of industrial effluents on the development and metabolism in amphibians. *Xenopus laevis* embryos exposed to sour water from a coal-gasification process exhibited high mortality and teratogenic effects (Dumont and Schultz, 1980). *Rana ridibunda* collected near the ironworks factory in Poland had significantly reduced metabolic rates that were inversely related to the distance from the mill (Pytasz, 1980). However, the clastogenic effect of pollutants on amphibians is hardly known. Chakrabarty *et al.* (1984) have reported a high degree of sister chromatid exchanges in the toads collected from a pesticide polluted area. Industrial waste-induced chromosomal damage has been noticed in *Rana temporaria* (Kraskowski *et al.*, 1986)

Compared to other metallic compounds, only a few information is available on the cytotoxic and clastogenic properties of cobalt compound. Cobalt is known to induce chromosomal aberrations in plants (Bhattacharya *et al.*, 1992) and are weakly

mutagenic in some *in vitro* cultured animal cells, bacteria and yeast (Jensen and Tuchson, 1990).

In the current study, an attempt was made to assess the clastogenic sensitivity of the chromosomes of the Indian bullfrog *Rana tigerina* to the river water contaminated with the industrial effluent. As the major contaminant of the river water was cobalt, a second set of experiments was also conducted by exposing the animals to the Cobalt sulphate solution, which contains the same amount of cobalt as in the effluent water.

Materials and Methods

Mature specimens of *Rana tigerina* weighing 40-60 gms were collected from uncontaminated water sources in the villages near Dabhoi. These animals were maintained in aquaterraria at 20-25 ^{0}C and fed cockroaches thrice a week. They were acclimatized to laboratory conditions for 15 days prior to experiments.

The effluent contaminated water was brought from the river Mini, which is about 4 km from Baroda city. Water samples were collected from the middle of the river, from a depth of about 2 feet. Analysis of the water sample was done by atomic absorption spectrophotometer.

Two series of experiments were conducted. In first series, the frogs were exposed to three different concentrations of effluent water. In the second series frogs were exposed to Cobalt sulphate solution.

Series A: No mortality was recorded within 20 days when frogs were kept in the 100% effluent water. Therefore, three different concentrations (25%, 50%, 75%) and two different treatment periods (3 days, 7 days, 14 days) were selected. Tap water was used to dilute the effluent water. A group of about 20 animals was maintained separately in tap water as control animals.

Series B: In this series, the frogs were exposed to the Cobalt sulphate solution that is having the same amount of cobalt in the effluent water. Only two concentrations

were selected for two different periods of treatments (7 days and 14 days). Control animals were maintained separately in tap water.

Preparations of mitotic chromosomes were made from bone marrow after *in vivo* colchicine treatment (Chapter 1). Sixty metaphase cells were analyzed from each animal. Statistical analysis of the data was done by equality proportion test and the value is considered to be significant when $Z \geq 1.96$

Mitotic index: Mitotic index was calculated using the following formula:
MI = (Number of cells in division/total number of cells counted)x 1000. Statistical analysis of data was done by Student's 't' test.

Results

Atomic absorption spectrophotometer analysis of the water sample is as given below:

Cobalt	-	398 ppm
Suspended solid particles	-	100 ppm
Total dissolved particles	-	5520 ppm
SO_4	-	3821 ppm
Cadmium	-	0.110ppm
Chloride	-	383 ppm

Cobalt was found as the major contaminant. High amount of sulphate was also found in this water, which had a low pH (3.8).

Data on the chromosomal aberrations in the bone marrow of the frog *Rana tigerina* induced by the effluent contaminated water are given in table 1. Table 3 shows the mitotic index recorded during the experiments.

Increase in aberrations was significant in all treatments. Maximum number of chromosomal aberrations were noticed in the animals treated for 14 days with 75% solution. In general, an increased number of centromere break and colchiploidy were recorded after the treatments. Multiple aberrations and fragmentations were more after the treatment with 75% solution for 14 days. Other types of aberration include

Table 1. Frequency of chromosomal aberrations induced in the bone marrow cells of frogs by Effluent water.

Dose and Treatment period	No. of animals tested	No. of cells analysed	Numerical Anomalies				Structural anomalies						Total No. of Anomalies	Percentage Aberrations	Z Value
			Hypodiploidy	Hyperdiploidy	Ployploidy	Total	Chromatid		Chromosome		Others @	Total			
							Gap	Break	Gap	Break					
Control	15	900	25	0	0	25	5	4	0	2	7	18	43	4.77	-
25%															
07 D	5	300	9	0	0	9	4	3	0	3	3	13	22	7.33	2.39
14 D	5	300	12	0	0	12	5	3	0	4	3	15	27	9.00	3.82
50%															
07 D	5	300	12	0	0	12	7	9	1	7	5	29	41	13.67	5.23
14 D	5	300	17	0	0	17	9	12	0	9	7	37	54	18.00	7.27
75%															
07 D	5	300	15	1	0	16	9	5	0	9	7	30	46	15.33	6.05
14 D	5	300	19	1	0	20	8	7	0	12	12	39	59	19.66	8.00

Result is statistically significant when Z≥1.96

@ = Acentric fragment, dicentric chromosomes or abnormal chromosome configuration

Table 2. Frequency of chromosomal aberrations induced in the bone marrow cells of frogs by Cobalt sulphate

Dose and Treatment period	No. of animals tested	No. of cells analysed	Numerical Anomalies				Structural anomalies						Total number of aberrations	Percentage Aberrations	Z Value
			Hypodiploidy	Hyperdiploidy	Ployploidy	Total	Chromatid		Chromosome		Others @	Total			
							Gap	Break	Gap	Break					
Control	15	900	15	0	0	15	6	6	0	4	10	26	41	4.55	-
50%															
07 D	5	300	5	0	0	5	2	4	0	1	2	9	14	4.66	0.08
14 D	5	300	7	0	0	7	1	3	1	3	5	13	20	6.66	1.44
75%															
07 D	5	300	7	0	1	8	2	2	0	2	4	10	18	6.00	1.01
14 D	5	300	8	0	0	8	1	4	2	3	3	13	21	7.00	1.66

Result is statistically significant when Z≥1.96
@ = Acentric fragment, dicentric chromosomes or abnormal chromosome configuration

Table 3. Mitotic index recorded in the frog bone marrow after the treatment with effluent water and cobalt sulphate. The data is presented as mean ±SE of 15 animals in control and 5 animals each in treatment groups.

	Control	25%		50%		75%	
	0	7 Days	14 Days	7 Days	14 Days	7 Days	14 Days
Effluent	2.88 ± 0.08	3.13^{NS} ± 0.15	3.61^{*} ± 0.18	3.24^{*} ± 0.06	3.43^{*} ± 0.06	3.95^{*} ± 0.08	4.19^{*} ± 0.11
Cobalt sulphate	2.48 ± 0.06	---	---	2.36^{NS} ± 0.09	2.08^{NS} ± 0.11	2.49^{NS} ± 0.16	2.57^{NS} ± 0.12

NS - nonsignificant; * Significant at 0.05 level

hypodiploidy, stickiness, centromere separation, and pulverizations (Plate 8). Some frogs, which were treated with 75% solution, developed lesions on the body especially, on the forelimbs and hind limbs.

Table 2 shows the effect of Cobalt sulphate on the chromosome of *Rana tigerina*. Cobalt sulphate could not produce any significant number of chromosomal aberrations in the test animal. Abnormalities recorded include stickiness, clumping other than a marginal increase in the breaks.

Table 3 shows the mitotic indices recorded during various treatments. Mitotic index was significantly increased in all the treatments in series-A, except for 7 days with 25% solution, while it was found marginally decreased when treated with Cobalt sulphate in most of the treatments.

Discussion

It was revealed from the first series of experiment that effluent water induced a significant number of chromosomal aberration in the bone marrow of the frog, *Rana tigerina*. Naturally, the heavy metal cobalt, the major component of the effluent, could be suspected as the cause of this effect. However, the results of the second series of experiments showed that Cobalt sulphate could not produce any significant number of chromosomal aberrations in the test animal.

In the first series, the mitotic index was found to be increased while it was significantly decreased in the second series. An increase in mitotic index was however, found in other experiments (Chapters 2 and 3) when the frogs were treated with the salts of heavy metals like mercury, nickel and lead. Such compounds are normally known to inhibit the cell division in mammalian systems (Sharma and Talukder, 1987). The hemopoietic system of amphibians is comparatively different from that of the mammals, and the bone marrow is less active in many of the amphibians. It seems that bone marrow gets activated due to the action of these compounds in blood. Frogs exposed to the effluent water for 14 days were developed lesions on the body, especially on the forelimbs and hind limbs. An injury on the limb is known to induce

Plate 8. Chromosomal aberrations induced by the effluent contaminated water in the bone marrow of the frog, *Rana tigerina* (all x 1075).

Figure 1. A hypodiploidy showing 21 chromosomes.

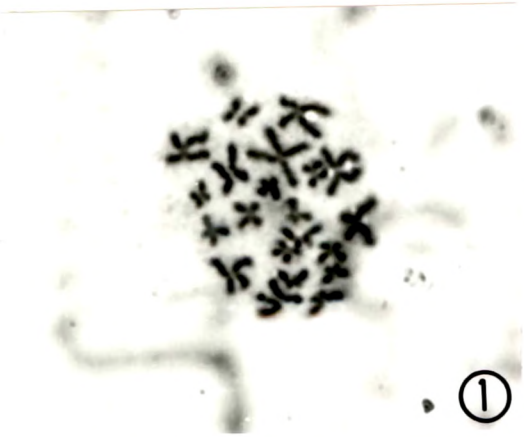
Figure 2. An isochromatid constriction near the centromere (arrow).

Figure 3. Separation of sister chromatids.

Figure 4. Colchiploidy.

Figure 5. Severe chromosomal breaks.

Figure 6. Extreme fragmentation of the chromosomes.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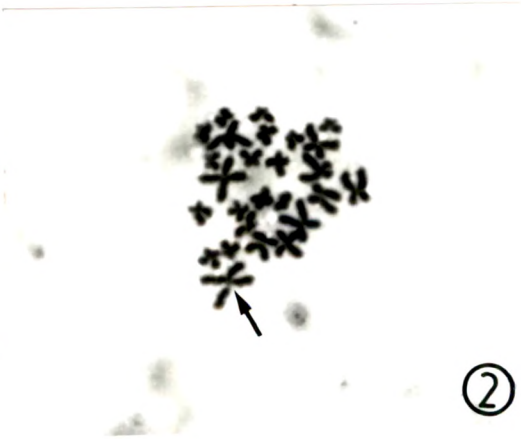
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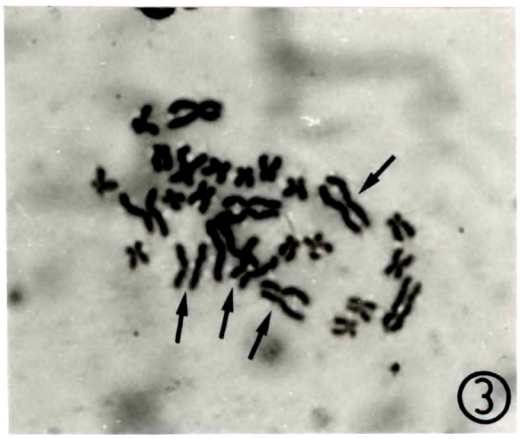
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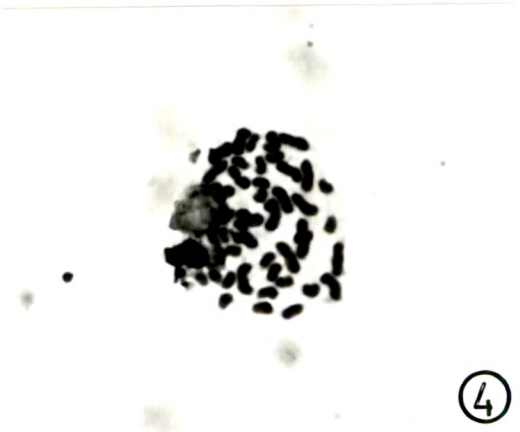
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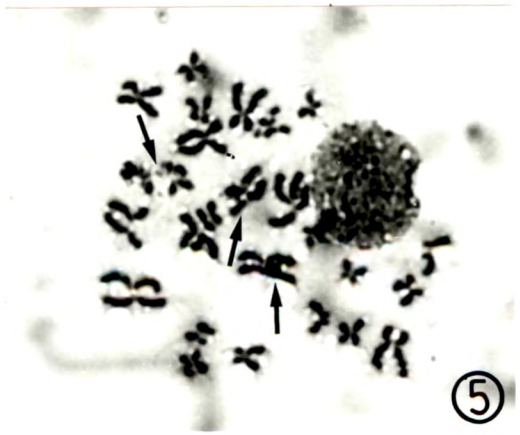
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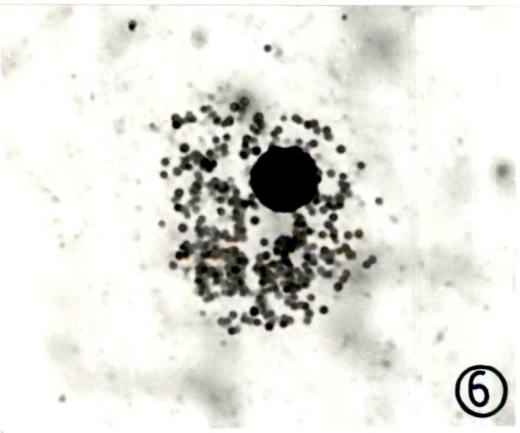
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the mitosis in the frog bone marrow (Haertel *et al.*, 1974). This could be a reason for the increase in mitotic index.

Soluble cobalt salts interfere adversely with cell division, bind irreversibly to nucleic acids in the cell nucleus (Jensen and Tuchson, 1990). A decrease in mitotic index observed in second series may be due to the antimitotic property of cobalt.

Centromere separation and colchiploidy (C-mitosis) were prominent among the aberrations while chromatid gaps and chromosomal breaks were minimum. Chromatid breaks were mostly found near the centromere. This situation was also observed in other experiments when frogs were exposed to heavy metals such as $NiCl_2$ and $HgCl_2$ However, an increased number of fragmentations and multiple aberrations were found when frogs were treated with 75% of effluent water.

It is apparent from the results that the mutagenicity of the river water was not solely due to the presence of cobalt. The effluent water had a low pH, which is an important factor that can be toxic by itself (Gosner and Black, 1957; Pough, 1976, Mc Donald, 1984) and can also influence toxicity of other substances (Hashimoto and Nichiuchi, 1981). Dissolved organic carbon levels are also important because metals can interact with organics and modify the toxicity (Porter and Hakanson, 1976). Mutagenicity can also be caused by the amount of other contaminants present in the water or more likely, due to the combination of these all factors (Sharma and Talukder, 1987). It has been also reported that heavy metals, especially under conditions *in vivo*, may act synergistically to enhance the mutagenicity of other mutagens present in the environment, although they themselves may be inefficient in producing chromosomal damage (Bauchinger *et al.*, 1976; O'Riordan *et al.*, 1978). Nevertheless, the present investigation shows a dose dependent increase in chromosomal aberrations with the induction of effluent water, and the genotoxic property of this water is beyond doubt.

Fishes are widely used to monitor the impact of pollution on aquatic environments. Amphibian could be used an indicator species to broaden this approach and might be particularly valuable for assessing the impact of pollutants on the genome of aquatic species.