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ORIGINAL ARTICLE

Studies on the Cytotoxic Effect of Oil Refinery Sludge on Root Meristem

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ABSTRACT

Cytotoxicity of the oil refinery sludge was studied using *Allium cepa* L. as a test plant. Onion roots were treated with different concentrations of pollutant for different durations along with controls. The high mitotic index and lower percentage of abnormality was very much evident in roots treated with diluted pollutants. The mitotic index showed decreasing tendency with parallel increase in pollutant concentrations. Binucleate, sticky anaphase erosion, C-metaphase, polyploidy, unequal distribution of chromosomes, spiral nature of chromosome and distortion of poles are some of the common abnormalities observed in all treatments. Both duration and concentration of the treatment influenced the cell division. The types and percentage of abnormalities confirmed impact of sludge generated by oil refinery on crop and other biota in the agro-ecosystem.

Key words: Oil refinery, pollutants, *Allium cepa*, Chromosomal abnormalities, Cytotoxicity.

Introduction

Rapid urbanization combined with industrialization has led to the generation and disposal of enormous amount of waste product to our environment [4]. Petroleum based industries including oil refineries are one of the major industrial sectors that generate tremendous amount of pollutants [10]. Crude oil is an oily mixture of different types of aliphatic, aromatic, polycyclic hydrocarbons (PAH) along with N, S, and O containing asulphants [5]. PAH is not only toxic but also highly carcinogenic [7]. A huge amount of refinery effluents is released to the environment, which pollutes both terrestrial as well as aquatic ecosystems. Sludge management in petroleum refineries is an area, which need immediate attention. Generation of variety of sludge in refinery can be minimized by taking appropriate steps, if not can damage the soil properties that might prove to be harmful for the farmers in their agricultural field nearby [6]. The higher concentration of these pollutants is likely to affect metabolic and cellular activities because of the presence of highly toxic and carcinogenic components in them [1].

Contamination of refinery sludge creates a distinct type of ecological habitat that invariably change the physico-chemical properties of soil leading to affect on germination of seeds, growth and development of plant lives. This oily sludge may completely eliminate the vegetation of a place hampering soil aeration, destroying rizosphere system including microbial populations. This brings a serious of disastrous changes in floral composition leading to habitat lost [8]. In this present investigation, therefore, an attempt was made to find out the possible toxic effects of effluents of petroleum-based industries on plant cells.

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Materials and methods

The refinery sludge was collected from the Noonmati Oil Refinery, which is located at about 10 km away from Guwahati city, Assam (India). Standard methods [2] were employed for the collection of sludge samples. The physico-chemical parameters of the samples were studied using standard methods [3].

Higher plants provide valuable genetic assay system for screening and monitoring environmental pollutants. *Allium cepa* L. has been considered as an excellent material for the assay of chromosome aberrations following chemical treatment. Their root meristem represents a normal proliferating plant pollution that is sensitive to changes in environmental conditions [16].

Healthy onion bulbs were selected to study the cytotoxicity. Bases of the bulbs were scraped to expose the fresh root primordial. These bulbs were than placed on the test tubes filled with distilled water in such a way that the bases of the bulbs were in contact with water surface. The bulbs, after they produced roots of about 1-2 cm length were taken out and placed on test tubes containing different concentrations of oily sludge [10%, 50%, 75% and 100% (raw sludge)] for 24, 48 and 72 hrs. A control set with dechlorinated tap water was maintained in all the cases. After treatment the root tips were excised and fixed in freshly prepared Carnoy's fixative (1:3 acetic acid and ethanol) for 24 hrs. Thereafter, the root tips were stored in 70% alcohol at 4^0 C until they were used. The root tips were stained in 2% Aceto-orcein to study the frequency of cell division and chromosomal abnormalities in different treatments. The frequency and types of chromosomal aberration were determined by examining about 1000 dividing cells from 10 root tips per treatment. The slides were observed under a compound light microscope (Leica ATC-2000). Toxicity to mitotic apparatus and karyotoxicity were also calculated.

Results and discussions

The physico-chemical characteristic of the oily sludge is presented in Table1. The sludge was acidic. The cytotoxic effects of oily sludge on somatic onion cells were estimated on the basis of changes in mitotic index and other induced abnormalities. The mitotic index and percentage of abnormalities observed are given in Table 2, 3, and 4. The mitotic index declined as the concentration of sludge increased. The percentage of abnormalities, however, showed opposite trend. The percentage of cell division and abnormalities induced varied in direct proportion to the sludge concentration and duration of exposure. Minimum cytotoxicity was observed in roots treated with 10% sludge while maximum effect was obtained in roots treated with raw sludge.

Both concentration and duration of exposure affected mitotic index. The root tips treated with sludge for 24 hrs induced lowest percentage of abnormalities while highest percentage of abnormalities occurred in roots treated for 72 hrs. Borah and Talukdar[9] have reported that higher concentration and longer duration of treatment of the castor seed extract depressed the mitotic index greatly. The sludge concentration in the range of 75-100% induced higher percentage of abnormalities and low mitotic index.

The root tips treated with lower concentration of sludge however showed higher mitotic index and lower percentage of abnormalities. Somashekar and Gurudev[19] recorded suppressive effect of industrial effluents on mitotic characters. Anucleation in considerable number of cells was an interesting finding of the present work. These cells might have developed due to cytokinesis without karyokinesis or due to the complete disintegration of the nuclear material. Considerable number of cells showed unequal distribution of chromosomes (Fig. 1G) that might be attributed to abnormality in spindle formation causing unequal distribution of chromosomes. Treatments of cells with 75% and 100% sludge produced cells showing nuclear disintegration. This aberration might have developed due to breaking of the nucleus. The nucleus gradually disappeared from the cytoplasm (Fig. 1D). Polyploid cells were encountered in all the treatments (Fig. 1A). Polyploidy might have occurred due to complete inhibition of spindle formation resulting in the failure of separation of chromatids and confinement of chromosomes within one nucleus [18]. The increase in chromosome number might also be due to the fragmentation of individual chromosome was also observed. These chromosomal fragments were discontinuities of chromatids. Fragmentations of chromosomes induce chromosomal mutation because of deletions and duplications, which may even lead to the death of cells [17]. Cytotoxic effects of several types of chemicals on the spindle apparatus were also reported by other workers in different plants [12, 18, 20].

In majority of the treated onion cells the telophase stage was observed without any sign of cytokinesis. The chromosomal halves after getting distributed to respective poles remained within the dividing cells instead of forming two daughter cells (Fig. 1B). This ultimately resulted in the formation of binucleate cells. Inhibition of cytokinesis and formation of binucleate cells were also reported in *Vicia faba* [15].

Abnormalities such as C-anaphase (Fig. 1H) and C-metaphase (Fig. 1I) were also observed. These abnormalities were reported to be induced by

Table 1: Physico-chemical parameters of raw sludge

Parameters	Raw sludge
pH	5.98
Conductivity (microsiemens/cm)	280.0
Oil and grease content (ppm)	345600
Organic matter %	54.94
Water holding capacity %	-
Ash content %	19.92
Metals (ppm)	
Pb	26.33
Mn	156.22
Ni	10.99
Zn	66.72
Na	3.80
K	0.90

Table 2: Effec of oily sludge on Allium cepa - 24 hrs treatment percentage of abnormalities and mitotic index.

Concentration of sludga	Total cells	No of cell in division	No of abberrated cells									Percentage of abnormality	Mitotic index
			Unequal distribution	Sticky anaphase	Polyploidy	Destruction of nucleus	Dislocarion of pole	C-anaphasa	C-metaphase	Binucleate cell	spiral chromosome		
10%	1000	496	03	07	02	05	07	02	02	-	-	5.64	49.6
50%	1000	432	07	11	06	11	09	03	05	-	-	12.03	43.2
75%	1000	391	04	12	08	12	05	07	07	01	03	15.08	39.1
100%	1000	360	06	13	10	16	10	09	10	03	03	22.2	36.0
Control	1000	547	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	54.7

Table 3: Effec of oily sludge on Allium cepa - 48 hrs treatment percentage of abnormalities and mitotic index.

Concentration of sludga	Total cells	No of cell in division	No of abberrated cells									Percentage of abnormality	Mitotic index
			Unequal distribution	Sticky anaphase	Polyploidy	Destruction of nucleus	Dislocarion of pole	C-anaphasa	C-metaphase	Binucleate cell	spiral chromosome		
10%	1000	401	02	07	06	12	05	02	02	-	-	8.97	40.1
50%	1000	371	04	08	08	14	04	05	03	01	01	12.93	37.1
75%	1000	322	10	11	18	29	09	06	07	01	03	29.19	32.2
100%	1000	307	19	28	20	26	20	11	14	02	04	46.90	30.7
Control	1000	517	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	51.7

Table 4: Effec of oily sludge on Allium cepa - 72 hrs treatment percentage of abnormalities and mitotic index.

Concentration of sludga	Total cells	No of cell in division	No of abberrated cells									Percentage of abnormality	Mitotic index
			Unequal distribution	Sticky anaphase	Polyploidy	Destruction of nucleus	Dislocarion of pole	C-anaphasa	C-metaphase	Binucleate cell	spiral chromosome		
10%	1000	393	07	11	14	20	08	05	05	02	01	18.57	39.3
50%	1000	327	13	09	26	30	10	07	07	02	05	33.33	32.7
75%	1000	279	20	23	28	36	30	11	10	02	07	59.85	27.9
100%	1000	228	25	32	40	46	33	13	15	03	10	95.17	22.8
Control	1000	513	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	51.3

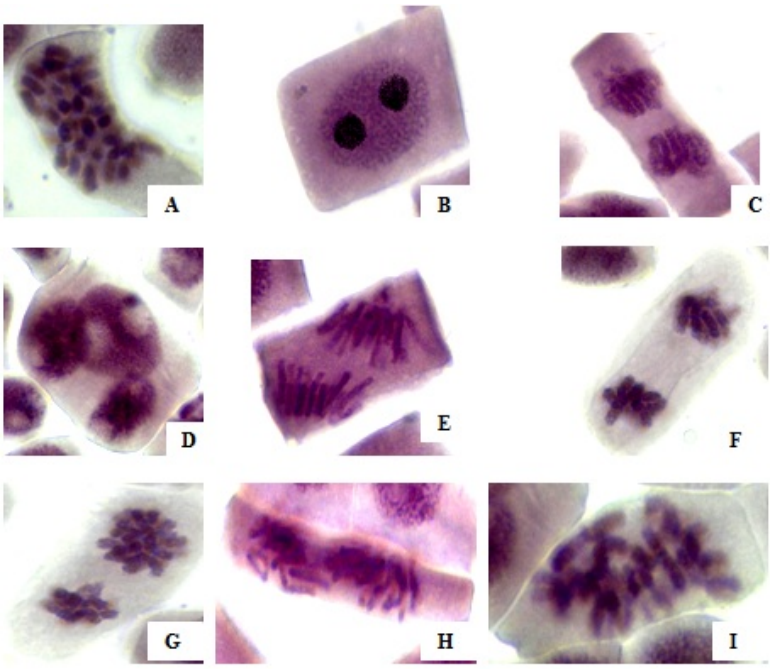

Fig. 1: (A) Cell showing polyploidy (B) binucleate cell (C) spiral nature of chromosome (D) Nuclear disintegration (E) Distortion of pole (F) sticky anaphase (G) unequal distribution of chromosome (H) C-anaphase (I) C-metaphase

treatments with chemicals like colchicines and organophosphorous pesticides [12, 14]. C-metaphase appeared due to inactivation of the spindle apparatus leading to the delay in the division of the centromere. The action of the chemicals like colchicines includes disturbance in protein and nucleic acid synthesis and antagonism between these substances [12]. This might also be as a consequence of inhibition of cell cycle in which chromosomal DNA is replicated but not distributed in its normal way [11]. The distortion of poles also observed (Fig. 1E).

Appearance of stickiness of chromosomes at anaphase was encountered in all the cells treated with different concentrations of sludge. The frequency of sticky anaphase was more in cells treated with higher concentrations of sludge than those with lower concentrations (Fig. 1F). The stickiness of chromosome at anaphase caused inability of normal movement of the chromosomes at anaphase. The stickiness of chromosomes was also reported in cells treated with organophosphorus pesticides [12] and industrial effluents [19]. Kaufma[13] reported that depolymerization of DNA and partial dissolution of nucleoproteins might lead to formation of sticky chromosomes.

Another interesting finding was the appearance of spiral nature of chromosome when cells were treated with 100% sludge for 72 hrs (Fig. 1C). The chromosome became spiral thread like structure due to uncoiling. It is important for understanding of the condensation of chromosomes particularly at metaphase.

The percentage abnormalities and the types of abnormalities encountered in the present study were interesting. On the basis of the results it may be concluded that the polluted oily sludge were capable of inducing variety of somatic cell abnormalities. Number of structural aberrations observed in the present study also indicated that the sludge acted as potential mutagenic agents. Therefore, it is necessary that in addition to routine physico-chemical and biological analyses, the industrial effluents should also be evaluated for the cytological damages caused by them to plant cells. The present study revealed that pollution caused by oil industries not only destroyed the balance in the ecosystem but also produced considerable genetic modifications in the flora growing in such habitats.

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