

## Bioassay Study of Biomedical Liquid Waste (Treated Effluent) of Bhopal City, Madhya Pradesh, India

Reeta Kori, Alok Saxena, Sandhya Mokhle, Vineeta Goutam,  
Ankita Kulshreshtha, Vandana Ramnani, Makhan Parmar

Central Laboratory, Madhya Pradesh Pollution Control Board, Paryavaran Parisar, E-5, Arera Colony, Bhopal,  
Madhya Pradesh, India

Corresponding Author: Ankita Kulshreshtha

### ABSTRACT

Biomedical liquid waste originated from hospital, laboratories & research centres has the potential to pose serious threat to the environment in terms of spread and contagiousness of infectious diseases to the system. Total fourteen hospitals were selected for this study. The study was conducted during April 2018 to March 2019. Monitoring of biomedical liquid waste (Treated Effluent) was performed as per guidelines of Central Pollution Control Board and bioassay test was performed as per BIS method, 6582: 2001. Biomedical waste may affected the aquatic life when it discharged in to the surface water. Fish are the primary bio indicator as they are very sensitive towards the pollution, so this study was based on toxicity test on fresh water *Brachydanio rerio* (Commonly known as Zebra fish). The observation based on 90% survival of tested fishes at 50% dilution (1:1 ratio of waste water to test water by volume) up to 96 hrs, that represented as toxicity factor one. Further increase in toxicity factor represents increase in toxicity found acute state. This study concluded that treated effluents from most of monitored hospitals of Bhopal city were found free from acute lethal toxicity.

**Key Words:** Hospitals, Biomedical Liquid Waste, Effluent Treatment Plant, Treated Effluent, Bioassay Test, Toxicity factor

### 1. INTRODUCTION

Bio-medical waste means any solid or liquid waste including its container and any intermediate product, which is generated during the diagnosis, treatment or

immunization of human beings or animals or in research pertaining thereto or in the production or testing. <sup>[1]</sup> Medical care is vital for our life, health and well being but the waste generated from medical activities can be hazardous, toxic and even lethal because of their high potential for diseases transmission. <sup>[2]</sup> Hospital liquid waste is wastewater generated from all activities of the hospital as medical and non medical activities from the operating, emergency & first aid, laboratory, diagnosis, radiology, kitchen and laundry activities. <sup>[3-5]</sup> Hospital liquid waste refer to infectious, non-hazardous and hazardous liquid waste with sufficient free liquid arising from dental, medical, nursing, pharmaceutical or similar practice, care, investigation, treatment that requires additional safety packaging to ensure safe transportation, treatment and storage. <sup>[6-7]</sup> In most developing countries, there is lack of technology, skills to implement and monitor hospital waste management programs. <sup>[8-10]</sup> Hospital wastewater includes macro- and micro-pollutants of wide concentration range from laboratories, research units, operation rooms, units, where medicine, nutrition solutions are prepared and polyclinics. <sup>[11-12]</sup> As a result of such consumption, the wastewater contains significant amounts of microorganisms, heavy metals, toxic chemicals and radioactive elements. Hospital originated wastewater is discharged to city sewage systems in many countries, treated together with domestic

wastewater and discharged to receiving environments. [13-14] Hospital laboratory wastewater is considered a mixture of pathogen microorganisms. The genetic structure of such microorganisms may be altered by the direct or indirect effect of wastewater components and lead to bacteria with high antibiotic resistance. [15] Many developed countries required disinfection of hospital wastewater before being discharged into sewage systems. Currently the most commonly used disinfectants are liquid chlorine, NaClO, ClO<sub>2</sub>, and O<sub>3</sub>. [16-17] The fact that hospital wastewater contains entero bacteria and enteric pathogens, poses a risk for public health. Today, the marked increase in antibiotic resistance of infectious agent pathogen bacteria seen both in nosocomial and community-acquired infections is one of the most important problems. [18] As a matter of fact, heavy metals pose threat to environment and human health since they are not biologically degradable pollutants and that they are movable pollutant sources. [19-20]

Bioassay is an assay designed to analyze any compound by use of a suitable biological system like animals,

tissues, microbes etc. It is defined as estimation or determination of concentration or potency of a physical, chemical or biological substance (agent) by means of measuring and comparing the magnitude of the response of the test with that of standard over a suitable biological system under standard set of conditions. [21] This study was based on bioassay test for analysis of toxicity of treated effluent from various hospitals of Bhopal city.

## 2. METHODOLOGY

### 2.1. Study Area

Bhopal city is the capital of Madhya Pradesh which is very well connected to all the corners of the country situated in the central part of India. Its latitude is 23°25'99 N and longitude is 77°41'26 E. Bhopal is also known as the "Lake City" for its various natural as well as artificial lakes and is one of the greenest cities in India. [22]

### 2.2. Monitoring Locations

Total fourteen hospitals of Bhopal city were selected for this study which are depicted in table no1 and shown in figure 1.

Table 1: Monitoring Locations

S.N.	Code	Monitoring Locations
1.	H1	Bansal Hospital Shahapura, Bhopal
2.	H2	Bhopal Fracture Hospital & Surgical Centre, Arera Colony, Bhopal
3.	H3	Bhopal Memorial Hospital and Research Centre, Bhopal
4.	H4	Chirayu Hospital, Bairagarh Kalan, Bairagarh, Bhopal
5.	H5	Gastrocare Liver and Digestive Disease Centre, Arera Colony, Bhopal
6.	H6	Government Shakir Ali khan(Gas Rahat) hospital, Bhopal
7.	H7	Hamidia Hospital, Royal Market, Bhopal
8.	H8	Indira Gandhi Mahila And Child Hospital, Bhopal
9.	H9	Jawaharlal Nehru Cancer Hospital Idgah Hills, Bhopal
10.	H10	J K Hospital & Medical Research Centre in Kolar Road, Bhopal
11.	H11	L B S Hospital, Motia Talab Road Bhopal
12.	H12	Kamla Nehru Hospital (Gas Rahat), Bhopal
13.	H13	People's Hospital Bhanpur, Bhopal
14.	H14	Swami Vivekanand Regional Spine Centre, M P Nagar, Bhopal

### 2.3 Monitoring and Analysis

Monitoring of treated effluents from effluent treatment plant of various selected hospitals was performed as per guidelines of Central Pollution Control Board and Bioassay test performed as per BIS method- 6582: 2001. [23]

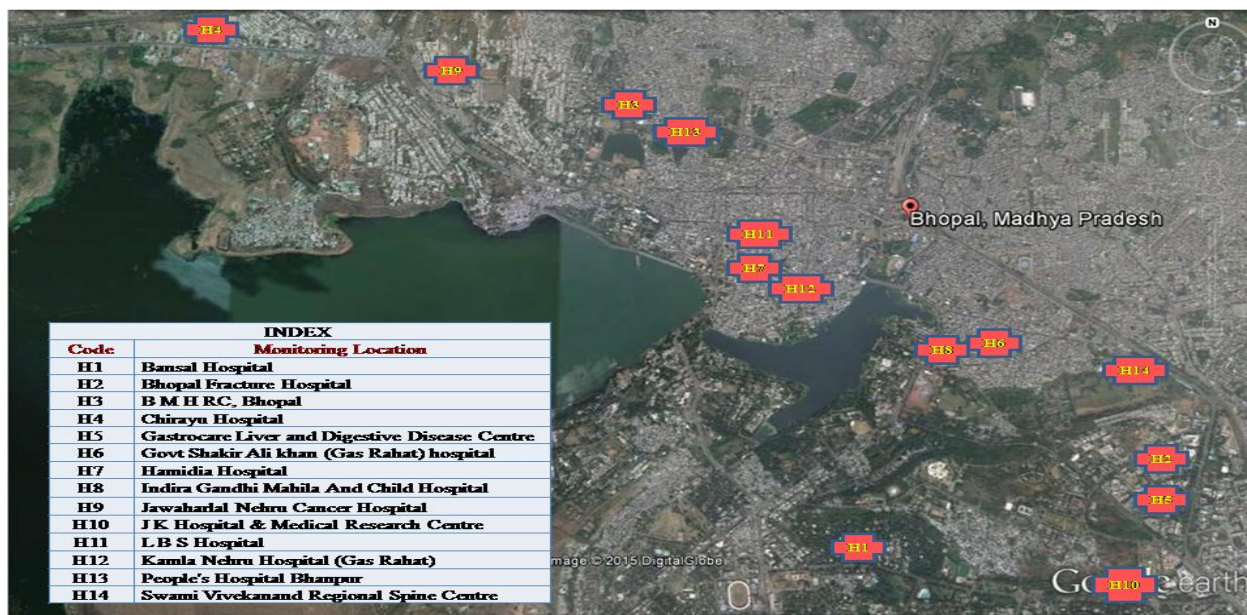


Figure 1: Monitoring Locations

### 3. RESULT & DISCUSSION

This study resulted by observations of bioassay test which are depicted in table no 2 to 16. In table no 2, toxicity factor (Tf) was found one during all four quarters observation of bioassay test of treated effluent from Bansal Hospital Shahapura, Bhopal.

Table 2: Bioassay test of treated Effluent from Bansal Hospital (H1), Shahapura, Bhopal

Quarter	Dilution Factor	Ratio of waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	6.98	7.02	5.5	3.0	100*
II (July-Sep 2018)	1	1:1	0	7.10	7.18	4.5	3.2	100*
III (Oct-Dec 2018)	1	1:1	0	6.82	6.86	5.2	3.1	100*
IV (Jan-Mar 2019)	1	1:1	0	6.85	6.92	5.3	2.8	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 3, toxicity factor (Tf) was found one during all two quarters observation of bioassay test of treated effluent from Bhopal Fracture Hospital & Surgical Centre, Arera Colony, Bhopal.

Table 3: Bioassay test of treated effluent from Bhopal Fracture Hospital & Surgical Centre (H2), Arera Colony, Bhopal

Quarter	Dilution Factor	Ratio of waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	#	#	#	#	#	#	#	#
II (July-Sep 2018)	#	#	#	#	#	#	#	#
III (Oct-Dec 2018)	1	1:1	0	7.25	7.33	5.0	2.9	100*
IV (Jan-Mar 2019)	1	1:1	0	6.95	7.08	4.8	2.5	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. . #Monitoring started after second quarter. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 4, toxicity factor (Tf) was found one during all four quarters observation of bioassay test of treated effluent from Bhopal Memorial Hospital and Research Centre, Bhopal.

**Table 4: Bioassay test of treated effluent from Bhopal Memorial Hospital and Research Centre (H3), Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	7.13	6.99	4.5	3.8	100*
II (July-Sep 2018)	1	1:1	0	6.94	8.18	4.8	2.5	100*
III (Oct-Dec 2018)	1	1:1	0	8.07	8.12	4.5	4.0	100*
IV (Jan-Mar 2019)	1	1:1	0	6.55	6.81	6.8	5.1	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 5, toxicity factor (Tf) was found one during all four quarters observation of bioassay test of treated effluent from Chirayu Hospital, Bairagarh Kalan, Bairagarh, Bhopal.

**Table 5: Bioassay test of treated effluent from Chirayu Hospital (H4), Bairagarh Kalan, Bairagarh, Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	7.68	7.72	5.8	3.1	100*
II (July-Sep 2018)	1	1:1	0	7.62	7.75	8.2	6.1	100*
III (Oct-Dec 2018)	1	1:1	0	7.02	7.64	5.4	3.9	100*
IV (Jan-Mar 2019)	1	1:1	0	7.11	7.28	6.9	5.3	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 6, toxicity factor (Tf) was found one during all four quarters of bioassay test observation of treated effluent from Gastrocare Liver and Digestive Disease Centre, Arera Colony, Bhopal.

**Table 6: Bioassay test of treated effluent from Gastrocare Liver and Digestive Disease Centre (H5), Arera Colony, Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	7.21	7.38	4.1	2.8	100*
II (July-Sep 2018)	1	1:1	0	7.16	7.19	4.5	2.9	100*
III (Oct-Dec 2018)	1	1:1	0	6.73	6.77	4.4	3.8	100*
IV (Jan-Mar 2019)	1	1:1	0	7.08	7.18	5.8	2.5	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 7, toxicity factor (Tf) was found two during first quarter and one during all other quarters of bioassay test observation of treated effluent from Government Shakir Ali khan (Gas Rahat) hospital, Bhopal.

**Table 7: Bioassay test of treated effluent from Government Shakir Ali khan (Gas Rahat) hospital (H6), Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	2	1:2	1	6.31	6.45	6.9	5.5	100*
II (July-Sep 2018)	1	1:1	0	8.23	8.38	7.1	6.3	100*
III (Oct-Dec 2018)	1	1:1	0	7.91	7.93	6.2	5.0	100*
IV (Jan-Mar 2019)	1	1:1	0	7.96	7.99	6.5	5.2	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume similarly 2 is for 1:2) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 8, toxicity factor (Tf) was found two during third quarter and one during all other quarters of bioassay test observation of treated effluent from Hamidia Hospital, Royal Market, Bhopal.

**Table 8: Bioassay test of treated effluent from Hamidia Hospital (H7), Royal Market, Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	7.16	7.31	6.3	4.8	100*
II (July-Sep 2018)	1	1:1	0	7.66	7.69	5.6	4.3	100*
III (Oct-Dec 2018)	2	1:2	1	6.56	6.78	6.2	4.1	100*
IV (Jan-Mar 2019)	1	1:1	0	6.85	6.93	5.8	3.6	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume similarly 2 is for 1:2) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 9, toxicity factor (Tf) was found one during all quarters of bioassay test observation of treated effluent from Indira Gandhi Mahila and Child Hospital, Bhopal

**Table 9: Bioassay test of treated effluent from Indira Gandhi Mahila and Child Hospital (H8), Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	6.96	7.18	6.6	3.8	100*
II (July-Sep 2018)	1	1:1	0	6.18	6.25	6.6	3.5	100*
III (Oct-Dec 2018)	1	1:1	0	5.61	5.71	7.1	6.8	100*
IV (Jan-Mar 2019)	1	1:1	0	7.23	7.31	7.7	6.5	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 10, toxicity factor (Tf) was found two during fourth quarter and one during all other quarters of bioassay test observation of treated effluent from Jawaharlal Nehru Cancer Hospital Idgah Hills, Bhopal.

**Table 10: Bioassay test of treated effluent from Jawaharlal Nehru Cancer Hospital (H9), Idgah Hills, Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	7.96	8.18	4.5	2.0	100*
II (July-Sep 2018)	1	1:1	0	7.73	7.81	5.8	3.2	100*
III (Oct-Dec 2018)	1	1:1	0	7.36	7.38	4.1	2.7	100*
IV (Jan-Mar 2019)	2	1:2	1	7.15	7.28	4.5	2.8	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume similarly 2 is for 1:2) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 11, toxicity factor (Tf) was found one during all quarters of bioassay test observation of treated effluent from J K Hospital & Medical Research Centre, Kolar Road, Bhopal.

**Table 11: Bioassay test of treated effluent from J K Hospital & Medical Research Centre (H10) Kolar Road, Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	7.23	7.36	5.8	2.5	100*
II (July-Sep 2018)	1	1:1	0	6.99	7.21	6.6	2.8	100*
III (Oct-Dec 2018)	1	1:1	0	7.11	7.19	5.6	2.3	100*
IV (Jan-Mar 2019)	1	1:1	0	7.28	7.35	6.5	2.9	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 12, toxicity factor (Tf) was found four during fourth quarter and one during all other quarters of bioassay test observation of treated effluent from L B S Hospital, Motia Talab Road Bhopal.

**Table 12: Bioassay test of treated effluent from L B S Hospital (H11), Motia Talab Road Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	7.09	7.18	6.2	3.8	100*
II (July-Sep 2018)	1	1:1	0	7.28	7.33	5.6	2.8	100*
III (Oct-Dec 2018)	1	1:1	0	7.21	7.26	4.7	2.5	100*
IV (Jan-Mar 2019)	4	1:4	3	6.51	6.66	5.5	4.5	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume similarly 2 is for 1:2, 4 is for 1:4) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 13, toxicity factor (Tf) was found one during all quarters of bioassay test observation of treated effluent from Kamla Nehru Hospital (Gas Rahat), Bhopal.

**Table 13: Bioassay test of treated effluent from Kamla Nehru Hospital (Gas Rahat) (H12), Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	#	#	#	#	#	#	#	#
II (July-Sep 2018)	1	1:1	0	7.46	7.48	5.8	3.2	100*
III (Oct-Dec 2018)	1	1:1	0	6.85	6.96	7.2	6.1	100*
IV (Jan-Mar 2019)	1	1:1	0	7.52	7.68	7.7	6.8	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. #Monitoring started after first quarter. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 14, toxicity factor (Tf) was found one during all quarters of bioassay test observation of treated effluent from People's Hospital Bhanpur, Bhopal

**Table 14: Bioassay test of treated effluent from People's Hospital (H13), Bhanpur, Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	1	1:1	0	7.25	7.28	4.1	2.5	100*
II (July-Sep 2018)	1	1:1	0	7.86	7.96	4.8	3.2	100*
III (Oct-Dec 2018)	1	1:1	0	7.47	7.52	5.2	3.1	100*
IV (Jan-Mar 2019)	1	1:1	0	6.16	6.25	6.8	5.1	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

In table no 15, toxicity factor (Tf) was found two during fourth quarter and one during all other quarters of bioassay test observation of treated effluent from Swami Vivekanand Regional Spine Centre, M P Nagar, Bhopal.

**Table 15: Bioassay test of treated effluent from Swami Vivekanand Regional Spine Centre (H14), M P Nagar, Bhopal**

Quarter	Dilution Factor	Ratio of Waste water to test water (by volume)	Parts of dilution water to be added to one part of Waste water (by volume)	pH (pH Unit)		Dissolve Oxygen (mg/l)		% Survival
				Before Test Start	After 96 hrs	Before Test Start	After 96 hrs	
I (Apr-June 2018)	#	#	#	#	#	#	#	#
II (July-Sep 2018)	1	1:1	0	7.28	7.31	5.1	2.5	100*
III (Oct-Dec 2018)	1	1:1	0	6.56	6.77	4.2	2.8	100*
IV (Jan-Mar 2019)	2	1:2	1	7.56	7.76	5.6	3.8	100*

Remark : \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume similarly 2 is for 1:2, 4 is for 1:4) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. #Monitoring started after first quarter. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

Summary of Bioassay test observations of studied Hospitals of Bhopal city is depicted in table no 16.

**Table 16: Summary of Bioassay test observations of studied Hospitals of Bhopal city.**

Code	I Quarter	II Quarter	III Quarter	IV Quarter
H1.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H2.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H3.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H4.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H5.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H6.	Toxicity factor (Tf) was found two*	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H7.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found Two*	Toxicity factor (Tf) was found one *
H8.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H9.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found two*
H10.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H11.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	The toxicity factor (Tf) was found Four *
H12.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H13.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *
H14.	Toxicity factor (Tf) was found one*	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found one *	Toxicity factor (Tf) was found two*

Remark: \*100% survival seen in observed Toxicity factor = dilution factor ( Tf 1 for 1:1 Ratio of Waste water to test water by volume similarly 2 is for 1:2, 4 is for 1:4 ) after 96 hrs. Standard Tf =1 represent test condition standard dilution water containing 100 mg/L Potassium dichromate, all fish should survive. Fish Species used for test: Zebra Fish (Brachydanio rerio) Fish size-30±5 mm and wt- 0.2 to 0.3 gm, Temp: 25±1.0°C.

The observation based on 90 % survival of tested fishes at 50 % dilution (1:1 ratio of waste water to test water by volume) up to 96 hrs, that represented as toxicity factor one. Further increase in toxicity factor represents increase in toxicity found acute state. The fish may not always die but their reproductive capacity, breeding, spawning and development are adversely affected. In aquatic toxicology fish have been widely and popularly acclaimed as a test species for evaluating the potency of toxicants to cause lethality (acute toxicity) or any other sub lethal responses, using selected behavioral biochemical or physiochemical and hematological responses. Bio medical waste effluents are complex and can be polluted by non-biodegradable end toxic organic compounds and are a serious threat to the environment. Fish are affected by toxic substances chiefly in following two ways: A- Epithelia -absorb toxic substances, getting damaged in this process, for instance, the gills stick together or get congested with mucus and get destroyed. B-

Besides the above immediate effects, harmful substances are adsorbed through the gills, skin or intestine, thereby impairing physiological functions. These effects may ultimately lead to the death of fish.

#### 4. CONCLUSION

Bio-medical waste is generated during the diagnosis, treatment or immunization of human beings, animals or from research activities pertaining there to or in the production or testing of biological fluids. It include wastes like human anatomical waste, animal waste, microbiology & biotechnology waste, discarded medicines & cytotoxic drugs, soiled waste, solid waste, liquid waste, incineration ash, chemical wastes etc. These wastes are very hazardous because of the potentially infectious in nature as it may pose a serious threat to human health, if its management is indiscriminate and unscientific. The study concluded that treated effluents from most of monitored hospitals of Bhopal city were found free from acute lethal toxicity



probably due to proper waste management practices adopted by the hospitals.

## 5. ACKNOWLEDGEMENT

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