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Full Length Research Paper

Genotoxicity Assessment of Hospital Effluents by Using Allium Cepa as Model Plant: A Comparative Study of Some Major Hospitals of Srinagar City, Jammu and Kashmir, India

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Abstract

Hospitals produce huge volumes of wastewater daily which contain a lot of toxic substances. Hence should be treated properly. Using onion (*Allium cepa*) root tip system, The current research work were carried out to assess the possible toxic effects of hospital effluents collected from main sewers from different hospitals, in Srinagar city, India. Before testing, *Allium* bulbs were kept in collected wastewater effluent for 48 hours at different concentrations. In addition, the *Allium* test was used to estimate DNA damage through comet assay.The results from the *Allium* test indicate that wastewater at 100% concentration from all the hospitals inhibits mitotic index and show schromosomal disruptions, but is not toxic at low concentration. In the comet assay, a statistical proliferation for DNA strand breaks was found only at higher concentrations of all the four samples. The samples were analysed by flame atomic absorption spectrometer for Zn, Pb, Cu, Ni, Cd and As, whose presence could somewhat be responsible for the toxicity of hospital waste water. The study revealed that the classical *Allium* cepa test can give a more reliable and accurate data when performed in combination with the comet assay, which is more rapid, easy and independent of mitosis. In addition, when hospital effluentmixes with the aquatic bodies, it causes serious impacts on aquatic organisms. It should be released after proper treatment into the environment in order to protect the ecosystem health from any potential adverse effects.

Keywords: Effluent, Environment, Comet Assay, Chromosomal Disruptions, Toxicity

INTRODUCTION

Hospitals generate a significant release of various toxic and nontoxic compounds into wastewater (Kummerer, 2002). All organisms that are exposed to hazardous waste are actually at a high risk, including the generators and also the waste handlers who manage the waste unscientifically. Despite the rising concern over hospital waste management, a very low care and attention has been given to toxic water effluents from health care establishments. Health care institutions utilise large volume of water per day, ranging from 400 to 1200 Lper day per bed (Deloffre-Bonnamour, 1995; CCLIN Paris-Nord, 1999)and also produce significant quantity of wastewater with a bulk of heavy metals and toxic chemicals. Thus hospitals, signifiesa sufficient release of various chemical substances intheir wastewaters and which may have an impact on the environment and human health. Indeed, some of the substances found in wastewaters are genotoxic and are suspected to be apossible cause of the cancers observed in the last decades (Jolibois and Guerbet, 2005 a-c). In order to determine the genotoxicity and mutagenicity of hospital effluents, *Allium cepa* has been widely used for chromosomal alterations through *Allium cepa* test and comet assay. Comet assay is attaining abundant importance to measure the DNA damaging ability of environmental chemicals because of its rapidity and sensitivity. Measurement of Comet tails is an important parameter as it represents free DNA fragments and shows damage in individual cells (Klaude et al., 1996). Alternatively the data obtained show good correlation with

the mammalian test systems may be more appropriate test model for Comet assay which has never been used for this purpose.

The present study is thus aimed at studying the mutagenic potential of the final discharges from hospitals. Four hospitals in Srinagar were studied, namely SKIMS, SMHS, LD AND JLNM. These sites were chosen because all these hospitals are located in prime locations of Srinagar and are amongst the major hospitals of Kashmir. The genotoxicity of wastewater at different concentrations of untreated water were examined in order to assess the toxicity of hospital waste water and examine the sensitivity of Allium root meristem cells against Comet assay to establish the utility of this system for both the assays. Amongst the sites chosen, SKIMS and SMHS hospital has a fully functional effluent treatment plant (ETP) whereas LD and JLNM hospitalsdo not have any treatment plant for their wastewaters Thus, this study reports the findings of genotoxicity tests for hospital effluents that are being released into the environment.

MATERIALS AND METHODS

Sampling of hospital wastewater

The samples from all the hospital sites (Figure 1) were taken twice in 6 months. The first sampling was done on 25th July 2007. The second sampling was done on3rd January2008.

Sampling site 1: The 814 bedded tertiary care hospitals, Sheri Kashmir Institute of Medical Sciences (SKIMS) in Soura area of Srinagar city was selected as site 3 and is located between the geographical coordinate's 34° 08´ N latitude and 74° 48´ E longitude. The institute is located 5 Kms away from the main city of Srinagar, and is located in a beautiful surrounding and overlooking, the Anchar Lake. SKIMS hospital has its own effluent treatment plant for wastewater generated in hospital due to patient and other treatment activities. Wastewaters are released into the effluent treatment plant where it is being treated and then released. Thus, the samples from this hospital were collected before the treatment process of the hospital.

Sampling site 2: The valley's top 700 bedded tertiary care hospital, Shri Maharaja Hari Singh (SMHS) hospital in Karan Nagar area of Srinagar city was selected as site 1 and is located between the geographical coordinate's 34°05´N latitude and 74°47´E longitude. It is a teaching hospital attached to the Government Medical College Srinagar, Kashmir. SMHS hospital also has a treatment system for the wastewaters generated due to hospital activities, but not all the waste water is being treated in the plant. Samples were therefore collected from the main sewer of the hospital, which receives the entire waste water from thehospital.

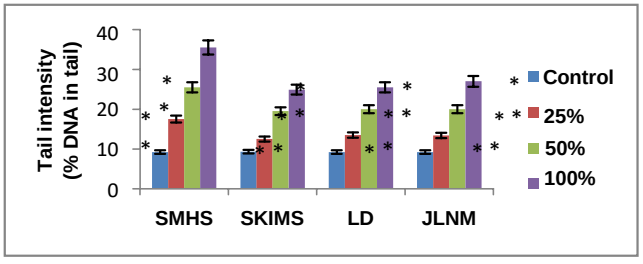

Figure 1. Effect of different concentrations (25%, 50% and 100%) of waste water effluents from different hospitals of Srinagar city on tail intensity of meristematic onion root cells by comet assay. Values are represented as mean ± SD and the data is based on triplicate experiment. Statistical analysis was done by ANOVA and significance was maintained at p<.05. **Highly significant (p<.001) and *significant (p<.05).

Sampling site 3: The valley's topmost 500 bedded Maternity hospital, Lal Ded (LD) is an associated hospital of GMC in Wazir Bagh area of Srinagar city. It is located between the geographical co-ordinates 34°04´N latitude and 74°48´E longitude. It caters the patients with Gynaecological and obstetrical ailments. It is the biggest maternity hospital of Kashmir valley. The hospital has a treatment plant but it is under construction. So the wastewater is collected from the main sewer of the hospital.

Sampling site 4: The 300 bedded Jawaharlal Nehru Memorial (JLNM) hospitals in Rainawari area of Srinagar city was selected as site 2 and is located in between the geographical co-ordinates 34°05'52''N latitude and 74°49'17''E longitude. It is a small scale hospital that caters to all the medical services of the health sector viz., Medicine, Ophthalmology, Surgery, Medical and Surgical, Maternity, Neurology, Emergency and Resuscitation, and Medical Emergency. The hospital does not have its own effluent treatment plant. The samples are collected from the main sewer where all the wastewater is being captured and flown outside the hospital premises.

The untreated wastewater samples from the effluent treatment plant were taken during the maximal hospital activity period (8:00 a.m-6:00 p.m). Samples were stored at 4degree C until tested. The samples were dilutes with tap water for making different concentrations. The undiluted sample was taken as 100% concentrated sample.

MATERIALS AND METHODS

Heavy metal analysis

For determining the heavy metal content in the hospital effluents, five liters of each collected water sample collected from different sites were concentrated on a sandy oven at 75 degree C until the volume reached 50 ml. Then 3 ml concentrated sulfuric acid (Merck, 98%) was added to each effluent sample and digestion was done by using digesdahl apparatus for few minutes. After digestion, hydrogen peroxide (10ml) was added and heated for oxidation. The samples were filtered by using 0.45μm What man filter paper just after cooling. Then deionised water was added to the filtrates for dilution to reach a final volume of 50 ml. The prepared samples were finally analysed by Atomic absorption spectrometry for estimating the heavy metals in the hospital effluents.

Mitotic index and chromosomal root aberration assay

To estimate the genotoxicity of the hospital effluents, untreated samples were collected at four selected hospitals of Srinagar city. All the samples were taken from the main sewer of the hospitals during the day hours (usually 7:00 am-7:00pm). The samples were filtered, acidified and stored at 4°C. The experiment was carried out according to method given by Fiskejo (1993).

For the *Allium cepa* test, four groups (one group of each hospital effluent) each with three bulbs were placed into four different hospital effluents at concentration of 25%, 50% and 100% for 72 h, and the control group was kept in tap water. When the roots reached to a length of 2cm,0.5 cm tips were collected and were fixed in aceto: alcohol (1:3) for 5min. A solution of 45% acetic acid and 1M HCl was used for hydrolysis and maceration. The staining ofthe chromosomes was carried out with 2% carmine in 45% acetic acid (Rank and Nielsen, 1993). After removing the rootcaps from well stained root tips, 1mm of the meristematic zones were dipped in a drop of 45% acetic acid on a clean glass slide and were squashed with the help of clean glass rod (Guerra and Lopes, 2002). Observation of prepared slides was performed under 1000x oil immersion light microscope and suitable pictures were captured as shown in Figure 2(A-E) for estimating the mitotic index and chromosomal aberrations for each hospital effluent concentration and control. For each concentration five slides were freshly prepared and 500 cells were examined per slide. For statistical analysis, one way ANOVA was performed with SPSS computer programme significant at p<0.05.The data value was expressed as mean with ± standard deviation (SD) for mitotic index and chromosomal aberrations was stated as mean with ± standard error

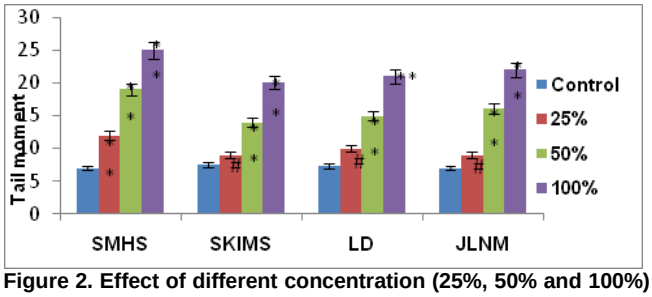

Figure 2. Effect of different concentration (25%, 50% and 100%) of waste water effulents from different hospitals of Srinagar city on tail moment on meristematic onion root cells by comet assay. Values are represented as mean ± SD and the data is based on triplicate experiment. Statistical analysis was done by ANOVA and significance was maintained at p<.05. **Highly significant (p<.001) and # non significant (p>.05).

Comet assay (single cell gel electrophoresis)

Root meristem cells of *Allium cepa* were exposed to similar concentrations as used for cytogenetic analysis. Comet assay (single cell gel electrophoresis) was evaluated by the method of Collins (2004). Fluorescence microscope with Olympus C5050 camera was used to analyse the comets. The percentage of DNA in tail (Tail intensity) and tail movement was evaluated by using open comet software.

RESULTS

Heavy metal analysis

Table 1 Mean values of heavy metals Co, Pb, Cr, Cd, Ni, and Zn and Hg, in the four hospitals effluents were found in the range of 1.64 Pb, ug/L, 23.25 Pb, ug/L, 13.24 Pb, ug/L, 2.42 Pb, ug/L, 14.89 Pb, ug/L, 1.42 Pb, ug/L, 33.14 Pb, ug/L, 334.8 Pb, ug/L and 6.68 Pb, ug/L.The maximum concentration of heavy metals was found in SHMS effluent followed by SKIMS hospital effluent due to high bed occupancy rate and the average daily flow of patients. However, the heavy metal content in LD and JLNM hospital was found to be less as compared to SMHS and JLNM hospital respectively. Overall, heavy metal concentrations in wastewater were in the following order: Fe > Zn > Cu > Cr > Ni > Pb > Hg > Co > Cd.

Mitotic index and chromosomal root aberration assay

We used the Allium test, which indicates damaged chromosomes or chromatids. The mitotic index value was high at 100% concentration of all the hospitals. The maximum mitotic index value was found at 100% concentration in SMHS effluent followed by SKIMS and the minimum value was found in LD and JLNM hospital effluent. The various types of chromosomal abnormalities produced by exposure with different concentrations of hospital effluents in meristematic cells of *A. cepa* are shown in (Figure 3 (A–I). These aberrations are metaphase disorientation, metaphase stickiness, anaphase stickiness, bridges formation at anaphase, C- mitosis and chromosomal breaks. The increase in the number of aberrations was very high at 50% and 100% concentration as compared to 25% concentration in all hospital samples. The results of the mitotic index are shown in (Table 2), and the chromosomal aberrations are shown in (Table 3).

Comet assay (single cell gel electrophoresis

The effect of different concentrations of waste water

Table 1: Shows the concentration of heavy metals in the effluents of Srinagar city.

Hospital name	concentration	Pb,ug/L	Cd,ug/L	Cr,ug/L	Zn,ug/L	Co,ug/L	Hg,ug/L	Ni,ug/L	Fe,mg/L	Cu,ug/L
SMHS	100%	34.9±2.02	2.8±0.1	16.5±2.32	542±51.12	1.1±0.12	11.2±1.6	19.5±1.92	1.9±0.02	36.03±1.5
SKIMS	100%	26.3±1.5	4.2±0.21	13.02±5.02	452±67.21	2.12±0.31	4.3±2.03	12.52±2.06	1.5±0.12	22.09±3.03
LD	100%	14.6±3.04	1.9±0.12	11.42±.4.21	402±190.2	1.34±0.02	4.04±1.29	16.21±2.91	1.21±0.10	35.04±4.91
JLNM	100%	17.2±2.01	0.8±0.31	12.03±3.12	341±131	2.02±1.5	7.21±1.42	11.34±0.03	1.08±0.31	39.41±3.32

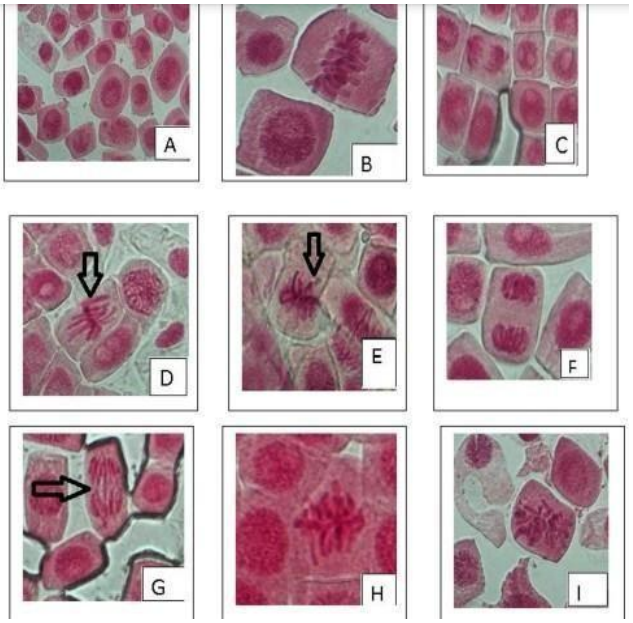

Figure 3. Some pictures of chromosomal aberrations observed in root tips cells of Allium Cepa A- Normal prophase; B- Normal metaphase; C- Normal anaphase: D- Disorientation at metaphase; E- Stickiness at metaphase; F- Anaphase stickiness; G- Anaphase bridges; H- C-mitosis; I- Chromosomal break.

Table 2: Shows the mitotic index at different concentration of hospital effluents. Level of significance:* significant, **highly significant.

Hospital name	concentration	Dividing cells	Mitotic index
	control	283	34.0±2.88
SMHS	25%	124	24.8±5.07
	50%	75.9	15.18±2.12
	100%	17.2	3.44±1.33
SKIMS	25%	135.4	27.08±6.07*
	50%	81.6	16.32±2.09
	100%	22.8	4.56±1.44**
LD	25%	201.3	40.26±8.02
	50%	104	20.8±3.42
	100%	42.9	8.58±1.02
JLNM	25%	175.6	35.12±2.23
	50%	97.8	19.56±1.83
	100%	51.4	10.28±1.02

Table 3: Shows the chromosomal aberrations at different concentrations of hospital effluents and control. Data in the table is expressed as mean ± SD. Level of significance: *significant, **highly significant.

Hospital name	Concentration%	Disorientation at metaphase%	Stickiness at metaphase%	Anaphase stickiness	Anaphase bridges%	C- mitosis%	Chromosomal break	Total cells showing abnormality
	Control	0.0 ± 0.00	0.2 ± 0.44	0.2 ± 0.44	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.44	0.6 ± 0.54**
SMHS	25%	3.6 ± 0.54	4.6 ± 0.89	5.6 ± 0.89	5.2 ± 0.44	2.6 ± 0.54	5.2 ± 0.44	26.8±0.73*
	50%	6.8 ± 1.09	11.2 ± 1.78	8.6 ± 1.51	8.0 ± 1.41	4.2 ± 1.09	8.0 ± 1.41	46.8±1.05
	100%	17.4 ± 2.30	18.4 ± 1.34	18.2 ± 0.44	17.4 ± 2.30	6.2 ± 0.44	16.4 ± 2.30	94±2.69**
SKIMS	25%	5.4±0.54	4.9 ± 1.92	4.2±1.30	3.0±0.70	2.6 ± 0.54	3.2 ± 0.83	23.3±0.34
	50%	5.0 ± 0.0	5.10 ± 1.00	9.4 ± 0.89	4.8 ± 1.09	4.2 ± 1.09	5.8 ± 0.44	34.3±1.02*
	100%	7.8 ± 1.09	6.2 ± 1.09	16.8 ± 1.30	7.4 ± 1.51	6.2 ± 0.44	7.8 ± 1.64	52.2±1.26**
LD	25%	2.0 ± 0.70	2.6 ± 0.89	2.6 ± 0.89	2.6 ± 0.54	2.0 ± 1.0	2.0 ± 0.70	13.8±0.94
	50%	4.8 ± 1.30	3.8 ± 0.54	2.8 ± 0.83	3.0 ± 1.0	2.4 ± 0.54	1.6 ± 0.54	18.4±0.73
	100%	7.6 ± 0.54	6.6 ± 0.54	5.0 ± 0.70	5.0 ± 0.70	4.4 ± 0.54	4.4 ± 0.54	33±0.92**
JLNM	25%	1.8 ± 0.83	3.6 ± 0.54	1.4 ± 1.67	2.2 ± 0.44	1.0 ± 0.0	2.2 ± 0.83	12.2±1.04
	50%	3.6 ± 0.54	4.8 ± 0.83	3.2 ± 0.83	1.8 ± 0.83	1.6±0.54	1.4 ± 0.54	16.4±1.91
	100%	4.4 ± 0.89	6.2 ± 0.83	4.0 ± 1.22	3.2 ± 0.44	2.0 ± 1.22	2.2 ± 0.83	22±1.12**

effluentscollected fromdifferent hospitals of Srinagar city on comet assay is shown in (Figure 1 and 2). A highly significant increase in tail moment and tail intensity was observed at 50% and 100% waste water effluents from all hospitals when compared with control. At 25% concentration highly significant increase in tail moment and tail intensity was observed in case of SMHS effluents. However, in case of SKIMS, LD and JLNM hospital effluents significant increase in tail intensity and non-significant changes in tail moment was observed at 25% concentration.

SMHS= SHRI MAHARAJA HARI SINGH HOSPITAL

SKIMS= SHERI KASHMIR INSTITUTE OF MEDICAL

SCIENCESLD= LALA DED

JLNM= JAWAHAR LAL NEHRU MEMORIAL HOSPITAL

SMHS= SHRI MAHARAJA HARI SINGH HOSPITAL

SKIMS= SHERI KASHMIR INSTITUTE OF MEDICAL SCIENCES

LD= LALA DED

JLNM= JAWAHAR LAL NEHRU MEMORIAL HOSPITAL

DISCUSSION

Cytotoxicity/genotoxicity of the effluent from the four different hospitals were evaluated using the *Allium cepa* test. At 100% concentration, mitotic index value was lowin SMHS hospital (4.46±1.27) followed by SKIMS (6.44±1.51), LD (9.42±1.12) JLNM (7.42±1.34) and LD (9.42±1.12) and the mitotic index of the control value (57.912±7.52) was found to be very high. This shows that SMHS effluent is much toxic when compared to other three hospital effluents. At 50% and 25% concentration, all the effluent samples shows increase in mitotic index value when compared to the control, but still shows some sort of toxic behaviour. The results, presented in (Table 2), demonstrate that there was a decrease in the mitotic index for each of the samples. This indicates, qualitatively, the presence of compounds with some degree of cytotoxicity present in the effluent. The cytotoxicity level can be determined by the decreased rate of the mitotic index. A mitotic index decrease below 22% of the control causes sub lethal effects on the test organisms (Antonsie-wiez, 1990), while a decrease below 50% usually has lethal effects (Panda and Sahu, 1985). This inhibition of mitotic activities may be correlated to the presence of cytotoxic substances Pb, Cd, As, Zn, Ni and Cu detected in wastewater.

Heavy metals are a major concern in the treatment of water and wastewater due to their toxic and other detrimental effects. The presence of heavy metals in wastewater samples is given in (Table 1). The estimated concentration of Zn was highest followed by Pb, Cu, Ni, Cd and As Allium test Table 2 shows that wastewater from all the four hospitals at 100% concentration inhibit root growth; decrease divisional frequency that were statistically significant when compared to control.

The most frequent type of chromosomal aberrations that were observed in our experiment are anaphase and metaphase stickiness, breaks in chromosomes, c-mitosis, bridge formation at anaphase stage and disorientation at metaphase plate. The stickiness in chromosomes may arise due to some faulty metabolic pathways in nucleic acids (Luca et al., 1987). The presence of chromosome stickiness reveals the fact that some chemicals are highly toxic in the effluent (Turkoglu, 2007). The anaphase bridge may be likely due to breakage of chromosomes. The disorientation of chromosomes, which was observed during the study, may be due to the effect of the toxic chemicals on the spindle fibers of microtubules and cause the misalignment of chromosomes at equatorial plate (Ozkan et al., 2009). Another chromosomal abnormality C-mitosis indicates the inhibition of spindle formation during metaphase. Presence of C-mitosis is asign of microtubule poison (Shahin et al., 1991). Chromosomal breaks were also observed in our study which may be due to clastogenic action of the toxic substances on DNA (Hobbs et al., 2017). The observed alterations clearly indicate the presence of toxic and mutagenic and/or genotoxic substances in the hospital samples. Alterations in the chromosomal structure are also observed at lower concentrations; however, the MI increases at lower concentrations (Table 1), showing that the cellular proliferation also increased. However, the more diluted the samples; less will be the genotoxic effect of the effluent on the living organisms. But, there may be bioaccumulation of the toxic elements such as heavy metals in the organisms. This demonstrates that the dilution reduces the cytotoxicity of the effluent, but, on the other hand, the residual cytotoxicity reinforces the idea that there should be effluent treatment plant in each hospital having the bed capacity of 50 beds so that the toxicity of the effluent will be removed to a great extent.

Similar bulbs were used for the evaluation of the DNA damage by the Comet assay *A. cepa* nuclei are very sensitive to the electric field, high DNA content, and less amount of the DNA in proliferating phase (Navarrete et al.,1997). In the current research work, the percentage DNA tail was statistically significant in all concentration starting from 25% whereas tail length (µm) and tail moment was statistically significant at the two higher concentrations (50% and 100%) when compared to control. It may be due to DNA damage induced by metals either by the introduction of oxidative DNA damage or by their interaction with DNA repair procedures (Hartwig, 1995). Wastewater from hospitals is cytotoxic, inhibits root growth, decreases mitoses and can cause DNA damage to the root meristems of onion. The effluents from all hospitals show genotoxicity in the form of increase in tail intensity and tail moment. The DNA breakage can be due to the heavily use of toxic chemicals and antibiotics being used in hospitals. Our results produced are in same agreement with one finding in which hospital wastes produce genotoxicity in the form of decreased mitotic index, increase in chromosomal damage

and increase in DNA tail intensity (Bhat et al., 2017)

CONCLUSION

Hospital wastewater is toxic in nature as revealed in the present study through bioassays such as allium test in combination with comet assay. Most of these toxic wastes have been revealed to cause hazardous effects on the living organisms. Based on the results we can concludethat Allium test can give a more reliable and accurate results when done in combination with comet assay, which is faster, simpler and independent of mitosis. Wastewater from hospitals is cytotoxic, inhibits root growth, decreases mitoses and can cause DNA damage to the root meristems of onion. The values are significant for cytogenotoxicity and are in agreement with similar bio-assays carried out by other authors elsewhere in the world. It is therefore necessary to implement strict regulatory enforcement laws on hospital effluent release into the environment for maintaining public health and safety.

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COMPETING INTERESTS

Authors have declared no competing interests.

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