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Toxicity study of Hospital Effluent using Plant, Animal and Bacterial Bioassays

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Abstract – In Hospital wastewater is a major source of pollution. It contains various potential hazardous materials including microbiological pathogens, radioactive isotopes, disinfectants, drugs, chemical compounds and pharmaceuticals. The components when discharged without any treatment can cause fatal effects on living organisms and plants. These toxic components can cause geno-toxicity and cyto-toxicity in living organisms. The study reveals the toxic effects of Hospital effluent using bio assays.

The analysis of various biomarkers gives us a clear indication of the cytotoxic and genotoxic effect of the pollutants. Plant systems like Allium cepa and Vigna radiata are historically used in toxicity studies. For this plant will be grown in different concentrations of Hospital effluent and compared with standard value. Various experiments to analyse the factors such as a root and shoot inhibition and chromosomal aberration shall be carried out. Haemolysis and the effect of these pollutants on bacterial cells (E-coli) shall also be determined.

Key Words: Bioassay, Chromosomal aberration, Haemolysis

1. INTRODUCTION

Hospitals represent an incontestable release source of many chemicals compounds in the environment due to laboratory activity or medicine excretion into wastewater. However the knowledge about the hospital wastewater toxicity is scarce and must be studied. Toxicity is often evaluated by means of biological tests, as well as by bacterial genotoxicity tests which do not require a prior knowledge of toxicant identity and/or physical– chemical properties. To evaluate the toxic/genotoxic risk of such complex mixtures, toxicity and genotoxicity tests employing microorganisms, plant cells and mammalian cells have been used alone or in combination with chemical analysis.

Bioassays are based upon the use of biological responses as detection system for biologically active substances. In the simplest form it is used to assay the presence (and concentration) of a particular substance by comparison with a known amount of the same substance. Both are procedures by which the potency or the nature of a substance is estimated by studying its effects on living matter.

Mutagenic activity of chemicals has been analyzed with different plant systems such as Allium cepa, Vigna radiata by exposing them in hospital wastewater over a particular period and the consequent potential growth inhibition is estimated. Both Allium cepa and Vigna radiata possess physiological properties (small size, high multiplication and vegetative propagation), which make them an ideal test system. Allium cepa aberration assay was utilized as a short term and cost effective indicator of toxicity in the routine monitoring. Optical density of E-coli bacteria is determined. Haemolysis assay is carried out in order to analyse the effect of hospital wastewater on human body.

2. METHODOLOGY

Hospital wastewater collection

The hospital wastewater used in the present experiment was collected from a famous Government hospital situated at the heart of Calicut city (Name can't be revealed due to some confidential issues). At this hospital no any proper treatment is provided for the wastewater and it is directed discharged in to the nearby water source. The wastewater collected was sealed in a plastic bottle and promptly transported to the laboratory for the experiments.

General chemical parameters of wastewater

The wastewater parameters examined are pH (APHA, 2017 Part 4500 H+), BOD (APHA, 2017 Part 5210), COD (APHA, 2017 Part 5220), Total suspended solids (APHA, 2017 Part 2540) and Oil and grease (APHA, 2017 Part 5520).

Treatment of Hospital waste water

For each seedling, there were 6 groups, and in the case of Vigna radiata consisted of at least 3 samples, whereas Allium cepa consist of only 1 sample. The negative control group was exposed to distilled water. Five treatment groups were exposed to hospital waste water of different concentration, by diluting the sample with distilled water (2%, 5%, 10%, 20%, 50%). All the experimental groups were kept in an incubator at 25+/- 1 ^{0}C.

Root and Shoot growth inhibition

The growth rate of both root and shoot of Vigna radiata was determined after 7 days. For accurate measurement of both root and shoot twain was used. Hence the lengths are determined.

In the case of Allium cepa the growth rate was estimated after duration of 10 days. Here also twain is used for accurate measurement. Length of both root and shoot is hence determined.

Fig-1 : Root and shoot tip preparation of Vigna radiata and Allium cepa

Chromosomal Aberration Test

A chromosome, disorder, anomaly, aberration, mutation is a missing, extra, or irregular portion of chromosomal DNA. It can be from an atypical number of chromosomes or a structural abnormality in one or more chromosomes. Chromosome anomalies usually occur when there is an error in cell division following meiosis or mitosis. There are many types of chromosome anomalies. Here in this study mitotic study is conducted since meiosis study would have been a longer and complicated step.

Bacterial Optical Density

Optical Density (OD) measurement of bacterial culture is a common technique used. Researchers have primarily relied on UV spectrophotometer to make these measurements, however the measurement is actually based on the amount of light scattered by the culture rather than the light absorbed. Using spectrophotometer to measure the optical density at 600 nm (OD600) of E-coli bacterial culture is used to measure the bacterial growth.

Haemolysis Assay

Hemolysis or haemolysis also known by several other names, is the rupturing (lysis) of red blood cells (erythrocytes) and the release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma). Hemolysis may occur in vivo or in vitro (inside or outside the body).

One cause of hemolysis is the action of hemolysins, toxins that are produced by certain pathogenic bacteria or fungi. Hemolysins damage the red blood cell's cytoplasmic membrane, causing lysis and eventually cell death.

3. RESULTS AND DISCUSSION

The level of Ph of tested wastewater is slightly alkaline and is between the desirable limit. BOD and COD values of the waste water were highly compared with the desirable limit, probably as a result of greater quantity of organic matter discharged. The total suspended solid is within the desirable limit. As expected the wastewater exhibit a little higher concentrations of oil and grease.

Table-1: Test results of General Chemical Parameters of hospital wastewater

SI. NO.	PARAMETERS	TEST METHOD	RESULT	DESIRABLE LIMIT AS PER (CPCB STANDARDS)
1.	pH	APHA,2017(Part 4500 H+)	7.19	5.5- 9.0
2.	BOD,mg/l	APHA,2017(Part 5210)	346.80	30.0
3.	COD,mg/l	APHA,2017(Part 5220)	1489.15	250.0
4.	Total Suspended Solids,mg/l	APHA,2017(Part 2540)	26.0	100.0
5.	Oil & grease,mg/l	APHA,2017(Part 5520)	32.40	10.0

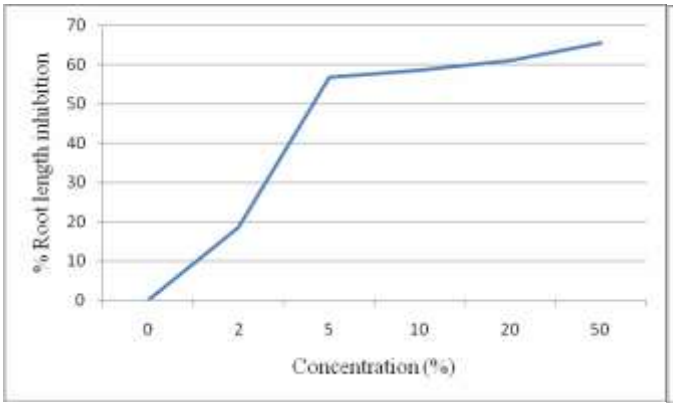

Chart -1: Root growth inhibition in Vigna radiata

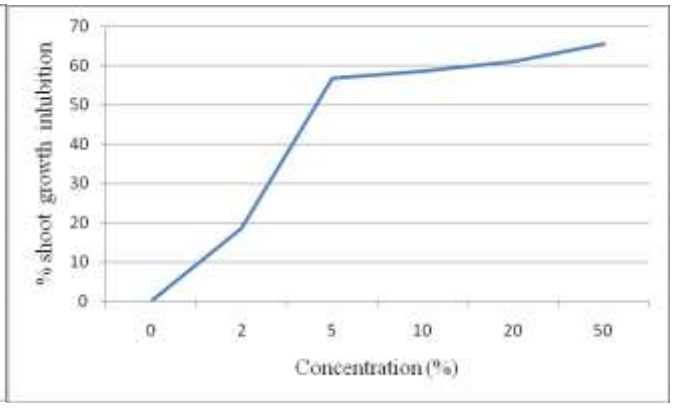

Chart -2: Shoot growth inhibition in Vigna radiata

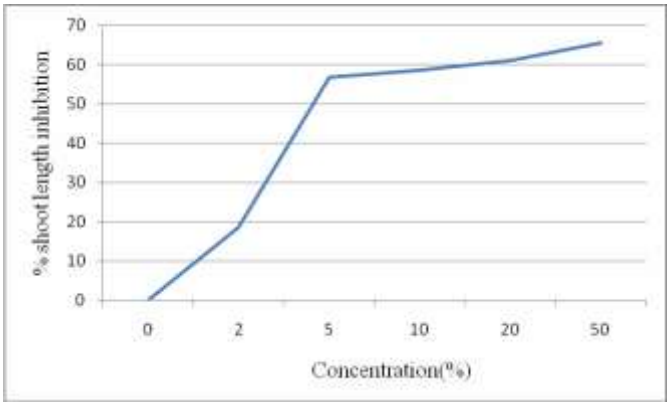

Chart -3: Root growth inhibition in Allium cepa

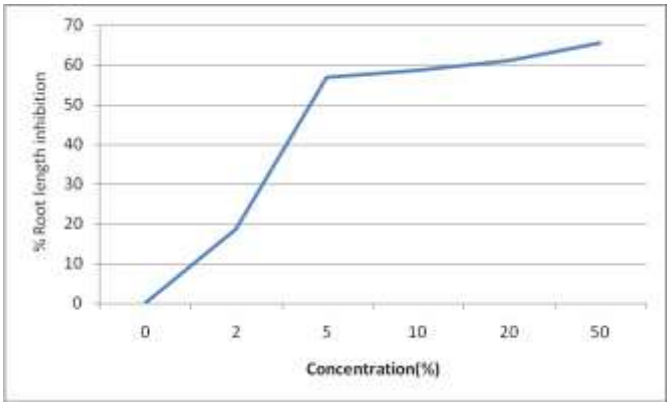

Chart -4: Root growth inhibition in Allium cepa

The results clearly indicated that with increase in concentration of toxic components, the growth inhibition percentage also increased. The observed growth inhibition can be attributed to the interference of toxicants with processes associated with root elongation in the zero contiguous to the meristems.

Genotoxic study of the hospital wastewater revealed how much the onion plants are affected internally. Chromosomal aberrations were scored for 10% concentrations of the onion meristematic cells at the root tips (as there lays the most actively dividing cells) along with the control sample. The aberrations were classified in the following categories: bridges, fragmented chromosomes, bi- nucleated cells, presence of micro-nuclei and many more. In this case, the stages of mitosis seemed to be disturbed and misarranged. Outcome of this study offer a useful guideline in evaluating the toxicological potential of Hospital wastewater even at very lower concentrations. These findings are of severe concern as the cell damage or the changes in the cell may be transmitted to subsequent generations. Meiosis study would have been a longer and complicated step using the shoot buds.

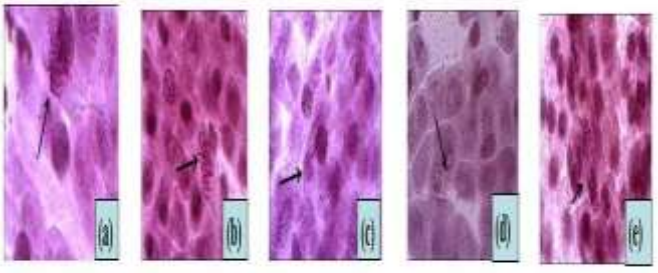

Fig-2: Chromosomal aberrations in Allium cepa (a) sticky anaphase, (b) disturbed metaphase, (c) bridge, (d) bi nucleated cell, (e) Fragmented chromosome

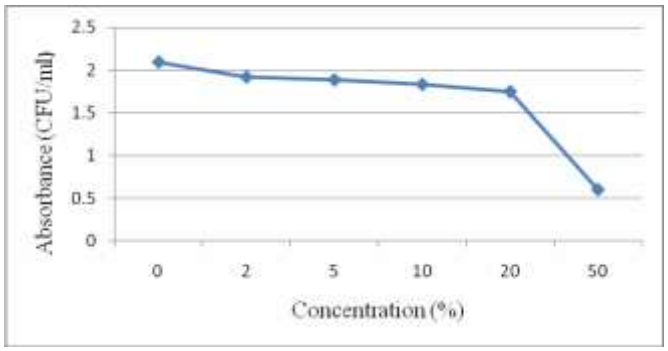

Chart -5: Optical density variation in E-coli bacteria The Optical density variation of bacteria in each

concentration of Hospital waste water shows a clear picture that it effects the growth of E-coli bacteria. So that we can conclude that the toxic components present in wastewater effects the cell division in bacteria. It is due to this reason that the rate of absorbance decreases from bacteria cultured in pure distilled water to that cultured in 50% wastewater.

In the case of SEM image of blood in control the cells are clearly viewed. Clear oval shape is seen. But where as in the case of the image of blood sample in 10% the cells appears to be disrupted. The cell wall seems to be broken. There is a slight variation in the shape also. Clear picture of Haemolysis is viewed.

Chart-6: SEM image of blood cell in 10% concentration

4. CONCLUSION

The results obtained provide evidence that Hospital wastewater is a genotoxic agent in plant, animal and bacterial cells, which imply that the wastewater could result in aquatic contamination even at dilute concentration and exposure of hospital wastewater might be a potential risk to induce damages of cytogenetic material for organisms. The result also suggest that Vigna radiata , Allium cepa, E-coli and Haemolysis cyogenetic bioassays can be used as efficient genotoxicity tests of hospital wastewater and the monitor of its pollution in the environment. The minimum facility requirements, and the simplicity and low cost of the procedure make the Allium cepa assay desirable for environmental monitoring. The study also demonstrated that the toxicity/genotoxicity bioassays should be an integral tool in the evaluation of wastewater toxicity prior to its release in to the environment. This study shows the usefulness of combining chemical analysis with cytogenetic methods to understand the toxicity of chemical pollutants and their influence on health.

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