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Materials and Methods
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MATERIALS AND METHODS

A. Selection of Hospital

Four hospitals were selected on the basis of number of beds in the hospital and for the purpose of convenience, the hospitals were designated as A, B, C and D. The study was conducted for 3 years starting from January 2010 to December 2012.

Information regarding the quantum of waste generated from each hospital was collected everyday either through personal visit or the hospital authorities were asked to furnish the details of biomedical waste generated through a questionnaire. In Mysore city, one private organization has been involved in collection and disposal of biomedical waste. The strategies adopted by this organization have been studied critically and information gathered is utilized for compilation of the thesis.

For the purpose of eliciting the required information the following study format has been prepared.

Format

Date of visit
Hospital Name
Doctor's Name and Specialization
Address
Tel. No.
Timings for visit
No. of patients treated per day
Type and quantum of wastes produced (Kg/gms)

- Needles, wastes, sharps
- Discarded glass wares (vials, ampules, bottles)
- Bandage cloth, Cotton, Plaster
- Excised tissues, placenta
- Other wastes.

All the 4 hospitals were visited every day for a period of three years starting from January 2010 to December 2012. For the collection of data pertaining to the source of generation of different types of biomedical waste, a good rapport was established and cooperation was sought from all the Heads of the Departments. The concerned staff were explained about the purpose of the study and requested to give free and frank opinion. The data thus collected was tabulated and subjected to analysis. Daily census record of the hospital was collected from the Administrative Record Office during the period of study.

As per the colour coded categorization of the biomedical waste, quantification of waste was done manually in the hospital daily during the entire period of investigation (Photo 5). The quantification was done both in terms of number of bags and the weight of the waste. Total number of bags and total weight of the waste was calculated by adding the data obtained daily. By this, the monthly quantification of biomedical waste was obtained as per the colour coded categorization in all the care centres during the course of the study.

Number of patients admitted (input of the hospital), number of patients discharged and number of patients in the hospital on any day was also recorded for all the 4 hospitals. By this, the bed occupancy data of all the 4 hospitals was collected and analyzed monthly.
B. Categorization and Quantification of BMW

Photo 5. Categorization of hospital waste collected in different coloured covers in plastic bins

Types of waste

1. Human anatomical wastes (human tissues, organs, body parts).
2. Waste sharps (needles, syringes, scalpels, blades and glass and such other things that may cause puncture / cuts).
3. Solid waste (items contaminated with blood and body fluids including cotton dressings, soiled plaster casts, beddings, other materials contaminated with blood, disposal items other than waste sharps such as tubings, catheters and intravenous sets).
4. Liquid waste (waste generated in the laboratory and washing, cleaning, house keeping and disinfecting activities) are the different types of biomedical liquid waste generated in the hospital.
The yellow covers contain infected cotton and dressings, plaster casts, beddings, items contaminated with blood and body fluids. Blue covers contain disposable items like syringes, catheters, intravenous sets, tubings, plastic bottles and contaminated plastic items.
Biomedical waste generated in the hospitals was segregated at the source and stored in colour coded bags as per the Biomedical Waste Management and Handling Rules, 2000. Each bag containing the waste was weighed separately every day during the course of study. The study was carried out for a period of 3 years (i.e., January 2010 to December 2012) to evaluate the change in the waste stream over time. To derive a representative sample, each category of waste generated has been weighed for all the 4 hospitals.

Photo 7. The quantification of biomedical waste

C. Characterization of Waste Water

Hospital waste water samples were collected in 1 liter can from the main underground drainage (UGD) points of the hospitals. While collecting the sample hand gloves and face masks were used. As soon as the sample was collected, it was taken for physico-
chemical analysis. Until the analysis was complete the samples were kept in the refrigerator. Once in 3 months the samples were collected from the 4 hospitals.

Waste water analysis is generally based on the three main categories of parameters i.e., physical, chemical and biological parameters. Electrical conductivity, pH, suspended solids, BOD, oil and grease, boron, sulphate and chloride are the parameters analyzed in the study. Therefore, the physico-chemical parameters considered for the present study include:

1. **Physical parameters**: Colour, odour, temperature, turbidity and suspended solids.

2. **Chemical parameters**: pH, BOD, COD, oil and grease, boron, sulphate and chloride are the chemical parameters considered for the study. Some metalloids such as boron, sulphate and chloride, were also studied and the data recorded. All the parameters were studied by APHA Standard methods (1998).

During the study, it was observed that the hospital waste water was not treated but was directly let into the UGD system. Physico-chemical parameters were analysed as prescribed by the Karnataka State Pollution Control Board (KSPCB). Sampling of waste water was done before it joined the UGD system. Four sets of samples of waste water were collected once in three months during the study period that is in January, April, July and October and analysed. The list of equipments used and methods followed for analysis are presented in Table 1.
Table 1. Analytical methods used for characterization of waste water

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Methods</th>
</tr>
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<tbody>
<tr>
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<td>Conductivity method</td>
</tr>
<tr>
<td>pH</td>
<td>Electrometric method</td>
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<td>Biological oxygen demand</td>
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<td>Oil and grease</td>
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<td>Chloride</td>
<td>Volumetric method</td>
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</table>

Physical Characteristics

i) Colour and odour

Historically the term ‘colour’ was used for the composition and concentration to describe waste water. It refers to the age of waste water which is qualitatively determined by its odour. Fresh waste water is usually light brownish grey in colour.

ii) Electrical conductivity

This was done by conductivity method as described by APHA standards (1998). Electrical conductivity is a measure of the capacity of wastewater to transmit electrical current. It is directly related to the concentration of ionized substances in the waste water. The cell constant of the instrument was set. The electrode was immersed in the waste water sample and the reading was recorded.
iii) Suspended solids

Suspended solids are used to evaluate the strength of waste water. Hundred ml of waste water sample was taken and filtered in evaporating dish with the help of Whatman No. 41 filter paper along with the residue. The filter paper was kept in the oven for 30 to 60 minutes. The dried filter was weighed along with the residue. Difference between the final and initial weights of filter paper divided by the volume taken is the suspended solids present in the waste water sample.

Chemical Characteristics

i) pH

pH was determined potentiometrically by a wide variety of pH meters, which are battery operated. They are equipped with glass and reference electrodes. It was standardized with standard buffer solutions before each measurement.

ii) Biological Oxygen Demand (BOD)

BOD is the measure of oxygen utilized by microorganisms during biological oxidation of organic matter contained in the waste water sample under specific experimental conditions.

Experimentally, it was determined by measuring the dissolved oxygen (DO) content of the waste water sample immediately after collection and after 5 days of incubation of the sample. The DO content of waste water sample at 1st day and at 5th day after incubation was determined. DO was determined by Winkler’s method. BOD was determined by multiplying the difference between the DO content of waste water sample by the ratio of volume of BOD bottle to ml of sample taken and subtracting the difference of 1st day DO of the distilled water (Blank) and DO at the end of the 5th day for the distilled water (Blank).
iii) Oil and grease

Oil and grease is extracted by adding petroleum ether under acidic condition in a separating funnel. After vigorous mixing, the ether supernatant mixture was separated in a clean and dry evaporating dish and the mixture was evaporated to get the residue. The difference between the weight of evaporating dish with residue and the weight of empty evaporating dish divided by volume of sample taken gave the oil and grease content in the wastewater sample.

iv) Boron

Boron was determined by taking 1 ml of waste water sample and adding 4 ml of curcumin reagent the mixture was heated till evaporation. Isopropyl alcohol was added and diluted up to 25ml. Using spectrophotometer the percentage of transmittance at the wavelength of 540 nm was recorded. Boron concentration was then determined by referring standard calibrated graph plotted against concentration versus percentage transmittance.

vi) Sodium Adsorption Ratio (SAR)

Trace amount of sodium can be determined by flame emission photometry at a wavelength of 589 nm. The extent of replacement of other cations with sodium was estimated by SAR. Calcium and magnesium were determined by volumetric titration with EDTA.

It was calculated using the formula:

$$\text{SAR} = \frac{\text{Na}}{\sqrt{\frac{\text{Ca} + \text{Mg}}{2}}}$$

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vi) Sulphate

Sulphate was precipitated in acetic acid solution with barium chloride so as to form a uniform suspension of barium chloride crystals. The absorbance of suspension was measured by photoelectric colorimeter. Sulphate concentration was determined by comparison of reading with standard calibrated curve.

viii) Chloride

Chloride ions are present in waste water as CaCl$_2$, MgCl$_2$ and NaCl. It was determined volumetrically by Mohr’s method by titrating against standard silver nitrate solution in the presence of potassium chromate indicator.

D. Characterization of Incineration Flue Gases

During the study, characterization of incineration flue gases was done thrice in a year during the study period, January 2010 to December 2012.

Photo 8. Pyrolytic incinerator used for the treatment of biomedical waste
Methodology for sampling and analysis of sulphur dioxide in ambient air
(Improved West and Gaeke Method, 2009)

Sampling

Place 30 ml of absorbing solution in an impinger and sample for four hours at the flow rate of 1 liter/min. After sampling, measure the volume of sample and transfer to a sample storage bottle.

Analysis

Replace any water lost by evaporation during sampling by adding distilled water up to the calibration mark on the absorber. Mix thoroughly, pipette out 10 ml of the collected sample into a 25 ml volumetric flask. Add 1 ml 0.6% sulphanic acid and allow reacting for 10 minutes to destroy the nitrite resulting from oxides of nitrogen. Add 2 ml of 0.2% formaldehyde solution and 2 ml pararosaniline solution and make up to 25 ml with distilled water. Prepare a blank in the same manner using 10 ml of unexposed absorbing reagent. After a 30 min colour development interval and before 60 minutes, measure and record the absorbance of samples and reagent blank at 560 nm. Use distilled water; not the reagent blank, as the optical reference.

Methodology for sampling and analysis of Nitrogen dioxide in ambient air
(Modified Jacob and Hochheiser Method, 2009)

Sampling

Place 30 ml of absorbing solution in an impinger and sample for four hours at the flow rate of 0.2 to 1 liter/min. After sampling, measure the volume of sample and transfer to a sample storage bottle.
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Analysis

Replace any water lost by evaporation during sampling by adding distilled water up to the calibration mark on the absorber and mix thoroughly.

Pipette out 10 ml of the collected sample into a 50 ml volumetric flask. Pipette in 1 ml of hydrogen peroxide solution, 10 ml of sulphanilamide solution and 1.4 ml of NEDA solution, with thorough mixing after the addition of each reagent and make up to 50 ml with distilled water. Prepare a blank in the same manner using 10 ml of unexposed absorbing reagent.

After a 10 min colour development interval, measure and record the absorbance of samples and reagent blank at 540 nm. Use distilled water not the reagent blank, as the optical reference. Samples with an absorbance greater than 1.0 must be re-analyzed after diluting an aliquot of the collected samples with an equal quantity of unexposed absorbing reagent.

A randomly selected 5-10% of the samples should be re-analyzed as a part of an internal quality assurance program.

Methodology for sampling and analysis of Suspended Particulate Matter (SPM) in ambient air (Gravimetric Method, 2009)

Sampling

Field Sampling - Tilt back the inlet and secure it according to manufacturers instructions. Loosen the faceplate wing nuts and remove the faceplate. Remove the filter
from its jacket and centre it on the support screen with the rough side of the filter facing upwards. Replace the faceplate and tighten the wing nuts to secure the rubber gasket against the filter edge. Gently lower the inlet. For automatically flow-controlled units, record the designated flow rate on the data sheet. Record the reading of the elapsed time meter. The specified length of sampling is commonly 8 hours or 24 hours. During this period, several reading (hourly) of flow rate should be taken. After the required time of sampling, record the flow meter reading, take out the filter media from the sampler, and put in a container or envelope.

Analysis

Filter inspection: Inspect the filter for pin holes using a light table. Loose particles should be removed with a soft brush. Apply the filter identification number or a code to the filter if it is not a numbered. Condition the filter in conditioning room maintained within 20-30°C and 40-50% relative humidity or in an airtight desiccators for 24 hours. Take initial weight of the filter paper (Wi) before sampling. Condition the filter after sampling in conditioning room maintained within 20-30°C and 40-50% relative humidity or in an airtight desiccators for 24 hours. Take final weight of the filter paper (Wf).