Disinfection Of Pathogens In Hospital Wastewater Using A Novel Plant Leave: Mimosa Pudica

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Abstract: Mimosa Pudica plant leaves was used to disinfect pathogens in hospital waste water. The physiochemical and biological characteristics of the waste water were determined. Three extracts of this leaves were prepared namely, fresh juice, aqueous and alcohol extracts. All the extracts showed a high potential to disinfect the pathogens in the waste water. The alcohol extract reduced the pathogens, DO, COD and BOD from (505 to 65) MPN/100ML, (32.7 to 4.04) M/L, (488.8 to 30.50) Mg/L and (266.2 to 16.67) Mg/L. The aqueous extracts reduced from (505 to 145) MPN/100ML, (32.7 to 6.88) Mg/L, (488.8 to 39.23) and (266.2 to 19.50) Mg/L while the Fresh Juice extract reduced from (505 to 263) MPN/100ML, (32.7 to 7.50) Mg/L, (488.8 to 50.56) Mg/L and (266.2 to 32.20) Mg/L respectively. The disinfectant effectiveness of the sensitive leave is in the order: alcohol extract > aqueous extract >Fresh juice extract.

Keywords: Hospital, Waste, Mimosa Pudica, Detoxification

Introduction

One of the challenges of developing countries like Nigeria and her likes is poor urban development and town planning management. Hospitals and factories are built anyhow near residential houses without drainage facilities. Suck away pits and well waters are dug without consideration of the flow direction of the underground waters. There will be a high probability of an outbreak of disease in the event of an increase in population as a result of urbanization and industrialization without a corresponding improvement in the health care and standard of living of the citizenry and the sanitary condition of the environment [1, 2] These situations have resulted to the increase in the number of hospitals and as such more volume of discharge of effluents into the environment. This indiscriminate discharge of hospital wastes without proper treatment have resulted to increased frequency of gastro enteric viruses in aquatic bodies [3] as well as increased risk of skin infections and other harmful diseases in humans [4]. The following microbial pathogens have been detected in untreated waste water: viruses, bacteria, protozoa and helminths [5, 6, and 7]. Among these microorganisms, bacteria is reported to be present in medium and high concentrations with consequent high infectious dose [8].

Other microorganisms found in hospital waste water [9] are coliforms, Escherichia coli, Clostridium perfringens, a faecal spore-forming bacterium, reported to be resistant to chlorine and also known to live longer in the environment [10]
Heavy metals are also not left out as contaminants of hospital waste water. The following heavy metals Arsenic, Cadmium, Chromium, Cobalt, Copper, Iron, Lead, Mercury, Silver and Zinc have been reported by [9, 10, 13]. There is also a tendency that these pathogen, if not well treated before discharging to the environment will contaminate the shallow well water scattered everywhere at the cities as

majority of the hospitals are close to residential houses. Hospital waste water is a biomedical waste and its treatment is different from other effluents treatment as it contains a lot of chemical, biological and pathogen in its mix [11].

Chlorine (free chlorine), ozone and ultraviolet radiation are the principal disinfectants used to treat wastewater. These materials are not only expensive but sometimes tend to toxify the receiving environment [12]. Therefore there is the need to source for a natural, low cost and nontoxic disinfectant.

In this research, Mimosa pudica plant leaves was used to treat hospital waste. The direct count method was applied in the determination of the effect of the sensitive leave on the concentration of the pathogen in the waste water.

Mimosa pudica also called sensitive plant, sleepy plant, action plant is a creeping annual or perennial flowering plant of the pea/legume family. It is often grown for its curiosity value: the compound leaves fold inward and droop when touched or shaken, and re-open a few minutes later. Like a number of other plant species, it undergoes changes in leaf orientation termed "sleep" or nastic movement. The foliage closes during darkness and reopens in light [14]

2.0 Materials and methods

Wastewater samples were collected from the three outlets of the Enugu State University of Science and Technology Teaching Hospital Enugu with sterilized dried plastic bottles. Due to low flow rate of the effluent, sample collection took three days. Each day, the samples collected were mixed, covered immediately and transported to the laboratory where it is stored in the refrigerator at about 4°C readiness for further analysis. The time between sampling and laboratory analysis was minimized to avoid degradation.

2.1 **Physicochemical Analysis of the Hospital Wastewater**

The physic-chemical analysis of the wastewater samples were carried out following the standard analytical methods for the examination of water and wastewater [15]. Temperature, total dissolved solids, dissolved oxygen, conductivity, and pH were determined at the sampling point using meters, while Chemical Oxygen Demand (COD), Biological Oxygen demand (BOD), nitrates and sulphates were analyzed in the laboratory with spectrophotometer.

Biochemical Oxygen Demand was determined by conventional methods using standard described by Ademoroti [15, 16]. Chemical oxygen demand (COD) was carried out using closed reflux method as described by Ademoroti [16]. .

Comparative analysis was carried out to determine whether there are variations between the point of discharge result and the Nigerian and world Health organization discharge standard limits. **Microbiological analysis of the Hospital Wastewater Inoculation and identification of microorganism**:

Pour plate technique, a type of pate count method was used in the determination of Total Bacteria in the sample. Stepwise serial dilutions of wastewater samples were carried out 1.0ml of wastewater sample was dispensed into sterile petri dish containing molten nutrient agar, Maconkey agar, Manitol salt agar and Sabouraud dextrose agar.

Each plate was swirled gently for easy mixing of the wastewater (inoculum) and the media, and incubated aerobically at 37°C for 24 to 48 hours. All the bacterial counts were counted and recorded as colony forming units per ml (cfu /ml). The bacteria was characterized using cultural identification .The biochemical tests were carried out to identify the isolates. The biochemical tests include; motility, catalase, indole production, citrate utilization, oxidase, methyl red and sugar utilization such as; glucose, lactose, sucrose, maltose, mannitol and inositol [17]

Determination of Coliform:

In the determination of the quantity of coliform, the most probable number (MPN) technique was used as described by [15]. The most probable number (MPN) of coliforms in the wastewater sample was estimated by the number of positive tubes corresponding with standard MPN statistical table and recorded as MPN/100ml.

Fungal count: For the fungal count, the sample dilutions were incubated in sterile *Sabouraud* Dextrose Agar plates supplemented with 0.05mg/ml chloramphenicol to suppress the growth of bacteria and spread with a sterile bent glass rod. The incubation period lasted for 4 days at room temperature. The fungal isolates were identified using their growth rate, colony morphology and microscopic morphological features.

Determination of E COLI and Salmonella

Escherichia coli: 100 mL of each sample was filtered through a 0.45 µm cellulose membrane filter (HA, Millipore, USA) that was placed on 5 mL of Luria-Bertani broth (1% tryptone, 0.5% yeast extract, 0.5%NaCl) and incubated at 37°C for 18/24 h.

Salmonella strains: 100 mL of each sample was filtered through a 0.45 µm cellulose membrane filter. Placed on peptone water, the cellulose membrane was incubated at 37°C for 18/24 h.

Determination of Heavy Metals

The samples were treated with approximately 3 mL of 1:3 HNO3: Deionized water per 250 mL sample before digestion was initiated. The Samples were collected and stored such that degradation or alteration is minimized. The EPA vigorous digestion method [15] was adopted.. The varying concentrations of the following identified toxic metals Arsenic (As), Cadmium (Cd), Lead (Pb), Mercury (Hg) and Chromium (Cr+6) in the wastewater effluents were determined by atomic absorption spectrophotometry (model AA340N)

2.2 Analysis of the sensitive plant leaves

2.3 Preparation of the sensitive plant leaves Extracts

Fresh leaves of Mimosa pudica were obtained from Amalla Udenu Local Government Area of Enugu State Nigeria. Three different extracts were prepared as described by [18].

Preparation of fresh juice Extract: The plant leaves were washed and rinsed with distilled water. (50 g) were made into paste with distilled water and ground using mortar and pestle. The paste was then diluted and filtered with a nylon cloth filter. The extract obtained was made up to 250 ml using distilled water.

Preparation of aqueous leaf extract: - The clean plant leave was sun dried, ground and filtered with 2mm sieve size. 100 g of leaf powder was dissolved in 500 ml of distilled water and kept for 8 hours at room temperature. It was filtered through a nylon cloth and centrifuged at 5000 rpm at 40C for 8 minutes. The extract was adjusted to the volume and stored in the refrigerator. [19].

Preparation of alcoholic extract: - 50 g of dried leaf powder were taken in separate container and mixed with 250 ml of methanol was added and kept for 24 hours with periodic shaking. The extract was then filtered through Whatman No.1 filter paper and filtrate was made up to volume and stored. [20].

2.4 Disinfections of Pathogens in the waste water.

Disinfection of the pathogens in the waste water was checked with the three prepared extracts of the sensitive plant leaves. 20 ml of each of the extract was added to 200 ml of waste water in three different 500 ml beaker. The forth beaker contains only waste without any extract and is kept as control. The beakers were incubated for 18 hours and serially diluted before plating in Nutrient agar plates to find out the total viable count. All the experiments were repeated three times and average reading were taken for analysis.

3. Results

Table 1: **Waste water result, national and international discharge standard limits**

s/n	Parameter	Unit	Wastewater discharge	National Standard (NESREA) [21]	International Standard (WHO) [22]
1	General appearance		Light	Clear	Clear
2	Color		130	5.15	5.125

3	Odor		Little	Unobjectionable	Absent
4	Turbidity	Ntu	630	10	10
5.	Temperature	ºC	29	Ambient	Ambient
6.	Ph		8.8	6.5-8.5	6.5-8.5
7.	Total Solids	Mg/L	820	1000	1000
8.	D .O	Mg/L	32.7	Minimum of 4	6.4
9.	C.O.D	Mg/L	488.8	30	30
10.	BOD	Mg/L	266.2	6.0	4.0
11	Conductivity	N/CM	1550.2	1000	1200
12	Sulphate	Mg/L	350.2	500	500
13	Nitrate	Mg/L	11	40	50
14	Alkalinity	Mg/L	5.51	Nil	Nil
15	Iron	Mg/L	0.04	0.3	0.5
16	Lead	Mg/L	0.07	0.01	0.1
17	Copper	Mg/L	0.14	1.0	1.0
18	Zinc	Mg/L	-	0.2	2.4
19	Coliform	MPN/100ML	250	100	100
20	E. coli	MPN/100ML	+ve	-ve	-ve
21	Total plate count	MPN/100ML	250	3	3

The physicochemical and biological analysis of the hospital waste water presented in table 1 shows that it has values of Dissolved oxygen (DO), chemical oxygen demand (COD), biological oxygen demand (BOD) and the pathogens higher than the permissible limit for the discharge of waste water stated by the national (NESREA) and international (WHO) standards. The high parameter values of monitoring standards of the hospital waste water is in accordance with the results of other researchers [9, 23, 24]

Table 2: Effects of the sensitive plant leaves on the pathogen content of the waste water.

S/N	Parameter	Unit	Control	Fresh Juice Extract	Aqueous Leave Ext	Ethanol extract	National Standard
1	Color		130				
2	Odor		Little	Little	Absent	Absent	Absent
3	Turbidity	Ntu	630	420	26	15	15
4	pH		8.8	8.2	7.8	7.5	7.4
5	TS	mg/L	820	810	805	800	500
6	DO	Mg/L	32.7	7.50	6.88	4.04	4.55
7	COD	Mg/L	488.8	50.56	39.23	30.50	60-90
8	BOD	Mg/L	266.2	32.20	17.50	16.77	30-50
10	Iron	Mg/L	0.04	0.04	0.03	0.03	0.03
11	Lead	Mg/L	0.47	0.45	0.35	0.22	0.21
12	Copper	Mg/L	0.24	0.22	0.20	0.19	0.18
13	Zinc	Mg/L	-	-	-	-	-

14	Coliform	MPN/100ML	250	136	91	55	25
15	E. coli	CFU/100ML	+ve	+ve	-ve	-ve	-ve
16	Total Plate Count	MPN/100ML	255	127	54	5	7

The effectiveness of the different extracts of the sensitive plant in the treatment especially pathogen disinfection is shown in table 2. The ethanol extract was found to be more effective than the fresh juice extract and the aqueous extract. The extract reduced the pathogens from 505 MPN/100ML to 32 MPN/100ML compared with aqueous extract and fresh juice extract that reduced the pathogens from 505 MPN/100ML to 60 155 MPN/100ML respectively. The reason may be as a result of the high solubility of alcohol that has the tendency to dissolve and absorb more bioactive materials that will inhibit the growth of the microorganisms. A similar high performance of the alcohol extract was obtained by the researchers [18]. The alcohol extracts also reduced the DO, COD and BOD from (32.7 to 4.04) Mg/L, (488.8 to 30.50) Mg/L and (266.2 to 16.67) Mg/L respectively which are all within the Nigerian standard for waste water discharge. The order of the effectiveness of the extract Mimosa Pudica in the disinfection of the hospital waste water is alcohol extract> aqueous extract > fresh juice extract.

4. Conclusions

Hospital waste water is a biomedical waste and its treatment is different from other effluents treatment as it contains a lot of chemical, biological and pathogen in its mix. There is a tendency that these pathogens, if not well treated before discharging to the environment will contaminate the shallow water wells scattered everywhere at the cities as majority of the hospitals are close to residential houses. Chlorine is the most common disinfectants mostly used for the treatment of pathogens but this chemical is very expensive and it also toxifies the receiving environment. It is therefore pertinent to source for natural and low cost materials for the disinfectant of pathogens. Three extracts of Memosa Pudica plant leaves namely: Fresh juice extract, aqueous extract and alcohol extract were used to disinfect pathogens in hospital waste water. All the extracts effectively inhibits the growth of the pathogens. The order of the effectiveness of the extracts of the sensitive plant leave is in the order alcohol extract> aqueous extract>fresh juice extract.

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