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Assessment of Pathogen Removal Potential of Root Zone Technology from Domestic Wastewater

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Abstract:
Root zone technology using constructed wetland (CW) for the treatment of domestic waste water was selected for the present study. Selected wetland unit was built during 2000 on the unused soil of Education College receiving the discharge of the sewage from the Ravindra Nagar (residential colony) at Ujjain (23^0 12' N latitude, 75^0 42'E longitude), situated in Madhya Pradesh, India. Results reveal that the mean reduction of indicator bacteria, total coliform and faecal coliform was 96% and 99% respectively in the inlet and outlet waste water from constructed wetland. Average reduction of waterborne bacterial pathogens, *Salmonella, Shigella* and *Vibrio* was 94%, 87% and 94% respectively through constructed wetland in three seasons e.g., rainy, winter and summer. Data collected from the experiments were statistically significant over their respective controls. The overall study strongly recommends the use of constructed wetland for treatment of domestic waste water for pathogenic bacteria, besides pollutant. Although it was a preliminary study, but originality of results are quite promising in terms of pathogenic bacterial removal from the wastewater.

Keywords: Coliforms, Constructed wetland, MPN, Pathogen

1.0 Introduction:
Constructed Wetlands (CW) are getting popularity due to their low cost eco-technology for wastewater treatment and also valuable for low income grouped human settlements that cannot bear the cost of conventional treatment systems (Reddy and Gale, 1994; Billore *et al.,* 2009). Domestic wastewater produced from the different origin contains organic solid waste (raw sewage), heavy metals, inorganic materials, sand, stones and number of pathogenic microorganisms and hence is the main source of diseases. Pathogenic organisms present in wastewater flourishaly grow in the presence of rich nutrient of the domestic waste. In addition, domestic waste water also contains certain group of bacteria and other microorganisms. A wide variety of microorganisms are helpful in the treatment of water biologically. These microbes are also varied in their nutritional requirements. Enterobacteriaceae is a group of bacteria having different pathogenic microbes e.g., *Escherichia coli* (including faecal Coliform), *Pseudomonas aeruginosa*, *Staphylococcus aureus,* faecal *Streptococcus*, some other forms of organisms such as *Protozoa* e.g., *Giardia lambia* and *Cryptosporium parivum, Balantidium*, in domestic wastewater. *Escherichia coli* bacterium is considered as an indicator of wastewater pollution. The most commonly occurring pathogens in the wastewater include strains of *Salmonella, Shigella, Leptospira*, intra-pathogenic form *of Escherichia coli*, *Pasteurella, Vibrio, Mycobacterium*, human enteric viruses, cysts of *Entamoeba histolytica* and hookworm larvae (Mitchell, 1971). There is a more than one process responsible for the reduction of pathogen population in wetland treatment systems, and essentially composed of combination of physical, chemical and biological factors (Gersberg *et al.,* 1984). Bacterial pathogens are removed by different processes such as sedimentation, chemical reaction, natural die-off and action of different and biologically active substances released by the plant roots and predation by water animals (zooplanktons). The contribution of each of the above ways is suggested to be a function of wastewater flow rates, nature of the macrophytes and type of the wetland (Mburu *et al.,* 2008). The majority of pathogens is come from the intestinal tracts of the human and enters into the environment

(water) and contaminates water bodies from these they enter to new hosts through ingestion (i.e., the faecal-oral route). Keeping these views in mind present study was conducted to study bacterial removal efficiency of root zone technology from domestic waste water.

2.0 Material and Methods:

2.1 Site description:

For the present study a field scale one celled horizontal subsurface flow (HSF) constructed wetland was selected. The selected wetland unit was built during 2000 on the neglected play ground of the Education College receiving the outfall of the sewage from the Ravindra Nagar residential colony in Ujjain (23^0 12' N latitude, 75^0 42'E longitude, mean sea level 515.45m), located in the central part of Madhya Pradesh state, India.

Fig. : 1 showing the height of reed grass

2.2 Sampling:

From the site 500 ml water samples were collected in sterilized glass stoppered BOD bottles. In the first step, the glass stoppered 500 ml bottle was washed thoroughly and rinsed with distilled water, for microbial analysis each dry bottle was rinsed with 0.5 ml Sodium Thiosulphate (10% solution) neutralized residual chlorine. Then stopper was loosely placed in the neck of bottle, wrapped in paper and sterilized at 121^{o}C for 15 min.

2.3 Enumeration of microorganism:

2.3.1 Total Coliform: Bacteria have been recognized as indicator of microbial quality of water. Coliform include aerobic and facultative anaerobic, gram-negative, non spore forming, rod shaped bacteria that ferment lactose with formation of gas and acid within 48 hrs at 35^o-37^{o}C. Multiple tube test was used for estimation of coliform group in water sample (APHA, 1990). Entire process is divided into three steps namely presumptive test, confirmative test and completed test.

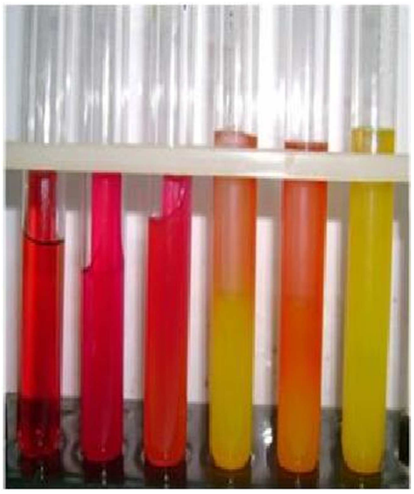

Fig. :1 Showing positive tube with acid and gas production

2.3.2 Faecal coliforms: This test was applied to different coliforms of faecal origin from coliforms of other sources. One loopful of growth from all positive presumptive tubes from the total coliforms MPN test i.e., Macconkey Bile Salt Lactose positive presumptive tubes were incubated in water bath at 44.5±0.2°C for 24 hrs. Gas production in the Durham tubes within 24±2hr and Faecal coliforms densities were calculated by comparing the combination of positive & negative tubes with MPN table (APHA, 1992).

***2.3.3 Salmonella* detection:** The sample was filtered using membrane filter and placed in a filtration unit. After filtration, the diatomaceous earth and filter paper was transfer to 50 ml Tetrathionate Broth (selective enrichment medium), and incubated for 24 hours at 35^{0}C and after proper growth sample was streaked on Brilliant Green Agar (2%) plates. Plates were incubated for 24 hours at 35-37°C. Pinkish white colonies with a red background isolated.

***2.3.4 Shigella* detection:** While most shigellosis epidemics are spread by contaminated food or by person-to-person contact, they may also be caused by contaminated drinking water. But methodology is qualitative and low in sensitivity (APHA, 1992). A selective enrichment medium to minimize accumulation of volatile acid by products derived from growth of coliform and other antagonistic organism using nutrient broth adjusted to pH 8.0 and incubates for 6 to 18 hrs at 35°C. Streak cultures at 6 to 18 hrs to Xylulose Lysine Dextrose Agar (HiMedia Lab.) plates to optimize *Shigella* recovery.

***2.3.5 Vibrio* detection:** The water samples from the inlet and outlet water of constructed wetland were enriched with alkaline Peptone Water (1% peptone, 1% NaCl, pH 8.4) blanks. Using appropriate dilution enrich sample were streaked on Thiosulphate Citrate Bile Salts Sucrose Agar (TCBS). TCBS Plates were Incubated for 24 hrs at 37°C and suspected *Vibrio cholerae* colonies appear yellow indicating sucrose fermentation.

3.0 Results and Discussion:

All five pathogenic microbial cell counts were done using methods described above (APHA, 1992). In case of highest total coliform reduction (98%) was during winter season, followed by summer season (96%) and lowest reduction (81%) was observed during rainy season **(Table: 1).** While in the case of faecal coliform highest reduction (98%) was during summer season, followed by winter season (91%) and lowest reduction (71%) was observed during rainy season **(Table:2)**. The mean reduction of indicator bacteria, total coliform and faecal coliform was 96% and 99% respectively.

Table 1: Average seasonal variation in Total Coliforms (CFU /100ml) with 't' values and their significant in treatment performance of constructed wetland water. Values in parenthesis represent standard deviation

Seasons	Inlet Water	Outlet Water	% Reduction	't' value	Remarks (significant)
Rainy	$1x10^6$ $(2.4x10^6)$	$1.9x10^5(4.8\ x10^5)$	81	1.014	**Not Significant**
Winter	$7.3\ x10^6(8.8\ x10^6)$	$1.1\ x10^6$ $(1.4\ x10^6)$	98	1.947	**Not Significant**
Summer	$12\ x10^7(1.4\ x10^8)$	$4.7\ x10^6$ $(4.3\ x10^6)$	96	2.354	**Significant**

Table 2: Average seasonal variation in Faecal Coliforms (CFU /100ml) with 't' values and their significant in treatment performance of constructed wetland water. Values in parenthesis represent standard deviation

Season	Inlet Water	Outlet Water	% Reduction	't' value	Remarks (significant)
Rainy	$5.1\ x10^6$ **(**$1.4\ x10^7$**)**	$1.5\ x10^6$ **(**$4.2x10^6$**)**	71	0.985	**Not Significant**
Winter	$6.6x10^6$ **(**$9.5\ x10^6$**)**	$6.2\ x10^5$ **(**$8.4\ x10^5$**)**	91	2.024	**Not Significant**
Summer	$10x10^7(1.4\ x10^8$**)**	$2.4\ x10^6$**(**$2.3x10^6$**)**	98	1.935	**Not Significant**

Table 3: Average seasonal variation in *Salmonella* (CFU /100ml) with 't' values and their significant in treatment performance of constructed wetland water. Values in parenthesis represent standard deviation

Season	Inlet Water	Outlet Water	% Reduction	't' value	Remarks (significant)
Rainy	$15\ x10^3$**(**$1.2x10^4$**)**	$2.2\ x10^3$ **(**$2.5\ x10^3$**)**	85	2.923	Significant
Winter	$2\ x10^3$ **(**$9.7\ x10^3$**)**	$1.7\ x10^3$ **(**$1.3x10^3$**)**	92	5.350	Significant
Summer	$8.7\ x10^4(1\ x10^5)$	$3.1\ x10^3$ **(**$4.2\ x10^3$**)**	96	2.391	Significant

Table 4: Average seasonal variation in *Shigella* (CFU /100ml) with 't' values and their significant in treatment performance of constructed wetland water. Values in parenthesis represent standard deviation

Season	Inlet Water	Outlet Water	% Reduction	't' value	Remarks (significant)
Rainy	$1.6\ x10^4$**(**$1\ x10^4$**)**	$1.4\ x10^3$ **(**$8.8x10^3$**)**	91	3.889	Significant
Winter	$1.4x10^4$**(**$6.8x10^3$**)**	$1.4\ x10^3$ **(**$9\ x10^2$**)**	90	5.258	Significant
Summer	$4.7\ x10^4\ (4.7x10^4)$	$6.9\ x10^3$**(**$9\ x10^3$**)**	85	2.381	Significant

Table 5: Average seasonal variation in *Vibrio* (CFU /100ml) with 't' values and their significant in treatment performance of constructed wetland water. Values in parenthesis represent standard deviation

Season	Inlet Water	Outlet Water	% Reduction	't' value	Remarks (significant)
Rainy	$1.5\ x10^3$ **(**$1.6x10^4$**)**	$1.6\ x10^3$ **(**$1.8x10^3$**)**	89	2.456	Significant
Winter	$2.8\ x10^4$ **(**$3.7x10^4$**)**	$1x10^3$ **(**$4\ x10^2$**)**	96	2.068	**Not Significant**
Summer	$7.8\ x10^4$ **(**$1.1x10^5$**)**	$4.6\ x10^3$ **(**$8.7x10^3$**)**	94	1.759	**Not Significant**

Salmonella bacterium reduction in all three seasons was assessed and it was found that 96% was decease in number of cells during summer season while it was lowest in rainy season (85%) as mentioned in the **Table: 3**. The highest *Shigella* reduction (96%) was during rainy season and lowest reduction (85%) was observed during summer season (**Table: 4**). The highest *Vibrio* reduction (96%) was during winter season, followed by summer season (94%) and lowest reduction (89%) was observed during rainy season **(Table: 5)**. Two sampling points (inlet/untreated and outlet/treated) designed in the present investigation.

Flowchart for water treatment:

Domestic wastewater

↓ In wetland system

Harmful effects are reduced

↓ Sub-emergent vegetation

Treatment/ purification

↓

A series of treatment processes (sedimentation, filtration, adsorption and various biological (including microbial) processes

↓

Treated water can be discharged in the water bodies

The elimination of pathogens is critically important in terms of public health and fitness. According to Awuah *et al.,* (2001), in developing countries these pathogens are major source of childhood death and major cause of mortality throughout the world. This has been pointed out that Root Zone technology originated from research concluded in Europe by Seidel and Kickuth at the Max Planck Institute in Plan, Germany starting in 1952 (Bastion and Hammer, 1992). Sohsalam *et al.,* (2008) conducted a study to remove pollutants from seafood processing waste water using constructed wetlands planted with six emergent species. Baskar *et al.,* (2009) also worked on *Phragmites australis* and found results similar to us which also supports our work. Application of constructed wetlands (CWs) in small town, district and area is now recognized as an accepted low cost eco-technology, especially beneficial as compare to costly conventional treatment systems (Mitsch, 1971; Reddy and Gale, 1994; Billore, 1998). Hua *et al.,* (2013), also performed the experiments related with constructed wetlands and their hydraulic mechanisms in reference with plant roots.

The active zone of constructed wetlands is root zone (or rhizosphere) of macrophyles growing there. Physic, chemical and biological processes take place that are induced by the interaction of plants, microorganisms, the soil and pollutants. There was significant removal of pollutants possible when contaminated water was passed through beds of reed plants (Seidel, 1978). For the treatment of sewage water in and around Pune and Bombay, using phytorid plants also carried out in the year 2013 (Karodpati and Kote, 2013). A preliminary study conducted to accept that wetland systems have excellent pathogen removal capability (Bavor and Andel, 1994). Biofilms present in the plant roots are believed to supply a more effective substrate than gravel for bacterial removal through various physical methods such as mechanical filtration, sedimentation, adsorption, die-off, predation and antibiotic excretion (Soto *et al.,* 1999; Karathanasis *et al.,* 2003)**.** Wetlands are therefore often called as kidneys of the Earth. Sedimentation and filtration are the two physical factors, which reduce pathogen in wastewater. Competition and natural death are event for pathogen killing. Removal of pathogen especially coliform contamination in the present study was in line with earlier report on 83-94% removal of enteric bacteria by surface flow constructed wetlands (Perkins and Hunter, 2000). The pathogen reduction mechanisms unique to reed beds include decrease by dense stand of reeds, predation by protozoa and exposure to antibiotic excretions from the roots of plants within the beds (Perkins and Hunter, 2000; Healy and Cawley, 2002). Wastewater may be contaminated with human, plant waste, other anthropogenic activities and animal pathogens. Pathogens found in wastewater include protozoa, parasitic worms, bacteria and viruses. Total or Faecal coliforms are generally only measured pathogen indicators in wastewater treatment wetlands (Preetha and Senthil, 2008).

Pathogens are removed in constructed wetlands during the passage of wastewater through the system by sedimentation and filtration. There are so many wastewater treatment methods available but their effectiveness in the removal of pathogens varies enormously (Cronk and Fennessy, 2001). A collection of physical, chemical and biological processes which are linked with and dependent upon each other, are involved in the alteration of nutrients and other substances and the wetland vegetation plays a significant role in these processes were reviewed by earlier researchers (Feachem *et al.,* 1983; Kadlec and Knight, 1996; Brix, 1997; Stottmeister *et al.,* 2003; Lee and Schloz, 2007; Gopal and Ghosh, 2008). Major design parameters, removal mechanisms and treatment performance have been reviewed (Cooper *et al.,* 1996; Vyamazal *et al.,* 1998; Kadlec, 2000; Vyamzal, 2005; Vymazal and Kropfelova 2008; Kadlec and Wallace, 2008; Villalobos *et.al.,* 2013). The presence of pathogens in wastewater is a widely recognized as indicator of water quality and the efficiency of the treatment method which is supported by earlier studies (Garcia *et al.,* 2003).

4.0 Conclusion:

The method of root zone in a constructed wetland is capable to remove indicator bacteria, Total Coliforms, Faecal Coliforms and pathogenic microbes including *Salmonella, Shigella* and *Vibrio* significantly and thus improve water quality**.** Constructed wetland System also provides aerobic conditions for microbial respiration to degrade organic matter due to photosynthesis by algae and oxygen diffusion from roots of the plant on bed. The overall study strongly recommends the use of CWs for treatment of domestic waste water for pathogenic bacteria, besides pollutants.

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