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# Occurrence and Biodegradation of Pharmaceutical Compound by Using Bacterial Consortium in Urban Lake of Madurai, Tamil Nadu, India

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**KEYWORDS**

Urban Lake, Sediment, Pharmaceutical compounds, Biodegradation, residual method.

**ABSTRACT:**

Pharmaceutical contaminants are emerging concern, as they are detected in many aquatic systems throughout the world because of their effects on biota and water quality criteria. The main source of contamination includes raw and treated pharmaceutical effluents, hospital waste, and excretion by livestock treated with antibiotics. The continuous exposure to pharmaceuticals increases the emergence of resistant bacterial strains which in turn cause unpredictable effects on human and animal. Hence, the present study evaluates the concentration of pharmaceutical compounds in the urban lake of Madurai city, Tamil Nadu, India and its biodegradation using bacterial consortium. The presence of pharmaceutical compounds were detected from water, sediment and fish samples by solid phase extraction cartridge (strata C18/X) and HPLC techniques. Three different drugs such as ciprofloxacin, ranitidine and sulfamethazole were detected in the samples and high levels of these compounds (43µg/L, 900 µg/L and 30 µg/L) were observed in fish, *Channa punctata* when compared to other samples. Two isolates, *Bacillus* sp., and *Clostridium* sp., were obtained from the sediment samples. Inhibition method was adopted to assess efficacy of the two isolates, as consortium, in degrading the pharmaceutical compounds. The consortium showed a maximum dégradationsof 43.57% after 28$^{th}$ day of incubation.

## Introduction

Rapid advancement in drug development and its usage, on one hand, relieves from diseases and ailments and, on the other hand, leads to severe health hazardous. Pharmaceuticals are complex molecules and are with high polar and hydrophilic nature than original compounds. Activities such as discharge of human excreta, disposal of unused drugs, livestock and farm practices lead to the entry of these pharmaceuticals in to aquatic bodies and dissolve in water [1-2] and dissolve in water [3]. The widespread use of these compounds made their presence in different environments *viz*., wastewater, surface water, groundwater, drinking water, sludge and sediments. The accumulation of these compounds pose serious threads to environment and living organisms.

Though the discharges from pharma industries are treated before release into the environment, the compounds are not completely removed before their entry into aquatic bodies because of improper legal

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implementation. Thus, these compounds find their way into bodies and subsequent accumulation. The accumulation and consequent transformation of these compounds into metabolites and conjugates results in high environmental risk [4]. COVID-19 pandemic enhanced the consumption of drugs and the concentration of paracetamol increased 170% and 198% in wastewaters compared to pre-pandemic periods in Greece [5] and in urban wastewater, worldwide [6-7].

Kuroda et al. [8] reported that the conventional wastewater process of removal of antiviral drugs are only 20% effective in removing drugs used to treat coronaviral disease. The pharmaceutical compounds significantly reduce fertility and endocrine disruption of environmental indicator cladoceran *Daphnia magna* and fish and increased resistance in bacterial pathogens [9]. The continuous exposure to pharmaceutical compounds leads to the emergence of drug resistant bacterial strains [10].

Various physical and chemical techniques are applied for the removal of pharmaceutical compounds and they are less efficient in the degradation but are expensive. Hence, efficient, eco-friendly and cost-effective technique to be identified for degrade pharmaceutical compounds by biological or microbial is considered as the one of the best options. Biodegradation of pharmaceutical compounds involves the conversion of the parent compound to metabolites by the action of microorganism in both aerobic and anaerobic condition [11]. Consortium of diverse microbial strains are further effective in degrading the pharmaceutical compounds [12]. Understanding the concentration of antibiotics in different environmental samples (surface water, sediment and biota samples) is a prerequisite and essential to evaluate the fate of antibiotics in aquatic environment [13] and its degradation. Hence, this works is aimed for evaluating the availability of pharmaceutical compounds in selected aquatic bodies and to assess the efficacy of microbial strains in the degradation of these compounds.

## *Material and methods*

### *Study area*

Vandiyur Lake (Latitude: 9° 54' 33.59" NLongitude: 78° 08' 26.40" E) located near K.K Nagar is one among the largest surface water bodies in Madurai City, Tamil Nadu, India. The lake is heavily loaded with toxic pollutants through the effluents discharged from various sources like chemical industries, bus terminal, markets and health industries. In the present study, samples were collected from three different locations, near Melamadai, Sundaram Park and Mattuthavani Market, along the banks.

### *Sample collection*

Water samples from three different sites were collected in sterile reagent bottles, brought to the laboratory. The sediment samples were collected from the bottom of the lake in sterile polyethylene bags. Two species of fish, *Catla cattle* and *Channa punctata* weighing equally were collected from the study sites with the assistance of help of local fisherman and transported to laboratory. All collected samples were immediately stored in ice at 4°C prior to analysis.

### *Physico-chemical Analysis*

Water quality parameters viz., pH, temperature, TS, TDS and chlorides were determined adopting the procedures outlined by APHA [14].

### *Sample preparation*

The collected samples were processed for the evaluation of pharmaceutical compounds.

A) Water Sample preparation: Water samples were filtered using Whatman No. 1 filter paper and 1ml of 50% sulphuric acid was added. The samples were then immediately brought to the laboratory for further analysis.

B) Sediment sample preparation: 2g sediment sample was taken in a centrifuge tube and to this 30ml of extraction buffer (15 ml of methanol, 0.1 M $Na_2EDTA$, 10 ml citrate buffer pH 5) was added and vortexed at 300 rpm for 20 minutes. The samples were ultrasonically extracted for 15 minutes and then centrifuged at 4000 rpm for 10 minutes. The process of extraction was repeated twice and the supernatant from three extractions were combined. Supernatant from each extraction process was collected and subjected to further analysis.

C) Fish sample preparation: The fish samples were washed with distilled water and placed on a clean tray. The bones and scales were removed, tissues were sliced and homogenized. 2 g sample was taken in a centrifuge

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tube and 1ml of $Na_2EDTA$ and extracting mixture (acetonitrile + citric buffer) were added. The contents were vortexed at 300 rpm for 10 minutes and were ultrasonically extracted for 15 minutes. The extract was then centrifuged at 4500 rpm for 10 minutes and the process was repeated twice. The supernatant from three extractions were collected and used for drug extraction.

***Extraction of drugs from samples using SPE cartridge***

A mixture of 500 µl of methanol and 500 µl of distilled water was used for conditioning SPE cartridges. The processed water, fish and sediment samples were loaded separately into individual cartridges. 10% methanol was used as washing solvent and samples were eluted. Eluates were steamed in water bath at 35 °C and samples were stored at 4°C for HPLC analysis.

***Screening of pharmaceutical degrading bacteria***

The soil samples collected from different locations were mixed and 1gm soil was suspended in 100ml sterile distilled water then stirred well and serially diluted. The dilutions were inoculated on to nutrient agar plates using sterile L-rod, incubated at 37 °C for 24- 48hrs. Individual colonies were picked up, sub cultured and stored in slant culture for further studies. Disc diffusion test was adopted to assess the sensitivity / resistance of the isolates against ciprofloxacin, ranitidine and sulfamethoxazole [15] and zones of inhibition were measured. The selected bacterial isolates were subjected to morphological and various biochemical tests [15] and Bergey's Manual of systematic Bacteriology was referred for characterization.

***Biodegradation test***

The bacterial isolates were grown on nutrient broth under rotary condition (130 rpm) at 30 **°C** over night. The cultures were centrifuged at 4300 rpm for 7 minutes and supernatants were discarded. The pellets were washed twice with saline solution and suspended in distilled water. The two isolates were mixed in 1:1 ratio and grown overnight as bacterial consortium. 100 ml mineral salt medium was taken in 500 ml flask and to this 0.3 mg of drug and 0.1 ml bacterial consortium were added to carry out biodegradation test and a control was maintained without consortium. The consortium was prepared by mixing the different isolates in 1:1 ratio and incubated overnight. The biodegradation test was carried out in 500 ml flasks containing 100ml of mineral salt medium. To this 0.3 mg of drug and 0.1 ml bacterial consortium were added. A control was maintained without the consortium and the flasks were incubated in shaker incubator at 130 rpm and 30 °C. One ml sample was taken on every 7$^{th}$ day, centrifuged for 20 minutes at 4300 rpm, supernatant was collected and analyzed using HPLC.

***Determination of drug degradation in HPLC***

The efficacy of the degradation of drugs by the consortium was determined by using HPLC (Agilent LC) with C-18 column with mobile phase (25% acetonitrile and 10% formic acid) at a flow rate of 1ml/min. 20 µl sample was injected and the peak was observed at 270 nm. The percentage of degradation was estimated using the following formula by substituting the peak area values.

$$\text{Percentage of drug degraded} = \frac{\text{Peak area of Test Sample x 100}}{\text{Peak area of Control}}$$

**Results**

***Physiochemical characteristics of the samples***

The physiochemical characteristics of both water and sediment collected from all three sites of sampling were analysed and the results were presented in Table 1.The pH values of all the three sites were higher than 7 indicating the alkaline condition of water. The TS, TDS and TSS values were higher in site II. The concentration of chloride was 9.99mg/L in site I and 19.99mg/L in site II &III. The pH of the sediment ranges from 7.5-8.8 and higher alkalinity is observed in site III compared to other two sites. The results of physicochemical characteristics reveal the contamination status of sites II and III in Vandiyur Lake.

***Detection of pharmaceutical compounds***

The collected water, sediment and fish samples of all the three sites were subjected to SPE (Solid Phase Extraction Cartridge) to extract the pharmaceutical compound and their concentration was determined using HPLC technique and given in Table 2 and figure 1. Three different classes of pharmaceutical compounds such as Ciprofloxacin, Ranitidine and Sulfamethazole were detected in the samples. Among them sulfamethazole showed low concentration in water compared to sediment and fish samples. Ciprofloxacin was detected

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only in *Channa punctata* whereas Ranitidine was detected in all the three samples at higher concentrations.

***Screening drug degrading bacterial strains and their characterization***

Among the bacterial strains isolated from sediment samples, four strains were tested for their sensitivity / resistance against ten different drugs and table 3 reveals the results. The results indicate that isolates 1 and 2 showed better resistance to selected drugs among the four strains and further degradation study was carried out with these two strains. The two selected strains were identified as *Bacillus* sp*.,* and *Clostridium sp.* by considering morphological, cultural and biochemical characteristics given in table 4.

***Degradation Analysis***

The effectiveness of the bacterial consortium in drug degradation was assessed by carrying out degradation experiments against the drug ranitidine. The degradation was carried out for 28 days. The degradation was determined by quantifying ranitidine on every 7$^{th}$ day by subjecting the sample for HPLC analysis. The analysis revealed a maximum of 43% degradation of ranitidine on 28$^{th}$ day of incubation (Figure 2).

**Discussion**

The levels of pharmaceutical compounds in the environment recently began to be more hazardous to the ecosystem [11]. The sewage, surface water and ground water were found to contain more than 80 pharmaceutical compounds. The concentration of the drugs differs based upon their usage. Gomes et al. [16] have reported the accumulation of hydrophobic pharmaceutical compounds in the sludge produced in WWTPs and soluble remains of hydrophilic compounds in the effluent. Thus, non treated and or inadequately treated effluents from industries and municipalities carry pharmaceutical compounds to aquatic systems. An attempt has been made to evaluate the concentration of drugs selected study site and to degrade with the help of bacterial consortium. The cooperative or synergistic effects among the bacteria in a consortium play a significant function and are more effective in the degradation of drugs [17]. In the present study, HPLC analysis revealed the presence of Ciprofloxacin, Ranitidine and Sulfamethoxzole in the samples collected from Vandiyur lake, Madurai, Tamil Nadu. The results further indicate the presence of sulfamethazole and ranitidine in all the samples but ciprofloxacin only in sediment and *Channa punctata*. Fishes serve as biomonitoring agent for xenobiotic pollutants as they accumulates hydrophobic organic compounds within the water column [18].

The ranitidine drug is present at higher concentration ranging from 0.948 µg/L to 900.08 µg/L in all the tested samples and this may be due to their less degrading ability of ranitidine. Ranitidine is a histamine H2 receptor antagonist which is commonly used for treating ulcer diseases, metabolised in the liver and about 70% is excreted in the urine as parent compound [19]. It is sold under the trade name Zantac and is one of the highly marketed drugs in many countries, around the world. Ranitidine finds a place in the top list among ecotoxicologically high risk compounds [20] and ranks 19 among recommended drugs [21].

Pharmaceutical compounds are not completely biodegradable, though they are designed for human / animal consumption. It is evident from the studies carried out in human waste samples that only a part of the drugs are metabolized and the remains are released into the environment especially water bodies [22]. In conformation to this report, the results of the present study also reveals that the water, sediment and inhabiting organisms especially fishes of Vandiyur lake are contaminated with drugs.

Ranitidine dissipation up to 90% was observed in 8hrs of continuous anaerobic-aerobic reactor-based degradation [23]. Sorption and dissipation of ranitidine in soil environment takes nearly 31days to degrade ranitidine in non-sterile and 62 days in sterile condition [24]. Mikeskova et al. [25], instead of single organism, used bacterial consortia to increase the rate of biodegradation of xenobiotics. The main role of using bacterial consortium is some bacterial species can remove the toxic metabolites released by the drug and others bacterial species can degrade the compound which the first species unable to degrade [26]. In our study, the degradation by bacterial consortium was estimated through HPLC and the recorded result shows 43.57% degradation of ranitidine on twenty eighth day of incubation. Similarly, the drug diclofenac took 30 days for its degradation [27] and naproxen 35 days [28].

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This study explores the possibility of degradation of drugs like ranitidine by bacterial consortium isolated from contaminated sites. Elevated degradation rate and complete degradation requires additional carbon sources and other supplements. Albuquerque et al. [29] reported that some bacterial population requires a specific substrate and more time to degrade the pharmaceutical compound. Supplementation of glucose [30] enhaced degrdation of diclofenac to 56% and complete degradation of diclofenac was yeilded by the addition of acetate [31]. When amino acids were added, sulfamethoxazole degradation rate was doubled [32] and 63% of ciproflaxcin degradation was achieved when Glycyl –L- glutamic acid, D- cellobiose and itaconic acid were added [33]. The results of the present study illustrates that mixed bacterial culture have the capability to degrade ranitidine. The study may further extend to evaluate the impact of supplements in enhancing the degradation of ranitidine.

***Conclusion***

The bacteria having the capability of degrading drugs were isolated from the sediment of lake soil and disc diffusion assay was performed to assess the efficiency of these isolates in degrading ciprofloxacin, ranitidine and sulfamethazole. Among four isolates two isolates showed resistance to ranitidine indicated by the absence of zone of inhibition. Considering the morphological and biochemical characteristics, the isolates were identified as *Bacillus* sp., and *Clostridium* sp. These two isolates were made into a consortium and tested for their degradation ability against ranitidine. The result revealed 43.57% degradation of ranitidine on 28$^{th}$day of incubation. The study concludes that the synergetic effect of microbial consortium may serve as an effective tool in mitigating pharmaceutical contamination. As the study confirms the source of pharmaceutical contamination of aquatic bodies, action plans have to be evolved to mitigate the contaminants by proper treatment of effluents and medi-wastes prior to discharge.

**Table legends**

**Table 1: Physiochemical parameters on target site in Vandiyur Lake**

**Table 2: Concentration of pharmaceutical compound in water, sediment and fish samples.**

**Table 3: Susceptibility of the bacterial strains to ten different drugs**

**Table 4: Biochemical test for identification of bacteria**

**Figure legends**

Figure 1: HPLC analysis for the pharmaceutical compounds: A) Ciprofloxacin B) Ranitidine C) Sulfamethazole

Figure 2: Degradation of target drug compound by bacterial consortium

**Table 1: Physiochemical parameters on target site in Vandiyur Lake**

| **Parameters** | **Site – I** | **Site - II** | **Site -III** |
|---|---|---|---|
| Water Temperature | 37 | 36 | 37 |
| pH | 7 | 7.5 | 8.5 |
| Total Solids(mg/l) | 0.8 | 48.8 | 1.6 |
| Total Dissolved Solids (mg/l) | 0.2 | 13.4 | 1.4 |
| Total Suspended Solids (mg/l) | 0.6 | 35.4 | 0.2 |
| Chloride (mg/l) | 9.99 | 19.99 | 19.99 |
| Moisture content | 16.42 | 11.2 | 10.34 |
| Sediment pH | 7.5 | 7 | 7.5 |

**Table 2: Concentration of pharmaceutical compound in water, sediment and fish samples.**

| Pharma ceutical | Water sample (µg/L) | Sediment sample (µg/L) | Fishsample (µg/L) |
|---|---|---|---|

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| compound | Site I | Site II | Site III | Site I | Site II | Site III | *C.catla* | *C. punctata* |
|---|---|---|---|---|---|---|---|---|
| Ciprofloxacin | ND | ND | ND | 5.0 | 9.0 | 23.0 | ND | 43.0 |
| Ranitidine | 0.948 | 28.01 | 165.06 | 70.028 | 300.02 | 408.21 | 20.008 | 900.08 |
| Sulfamethazole | 0.947 | ND | ND | 7.002 | 2.44 | 8.24 | 21.03 | 30.82 |

***ND- Not detected**

**Table 3: Susceptibility of the bacterial strains to ten different drugs**

| **Antibiotics** | **Isolate 1** | **Isolate 2** | **Isolate 3** | **Isolate 4** |
|---|---|---|---|---|
| Streptomycin | R | R | S | S |
| Tetracycline | R | S | R | S |
| Ampicillin | R | R | R | R |
| Erythromycin | R | S | S | S |
| Gentamycin | S | S | S | S |
| Penicillin | R | R | R | R |
| Diclofenac | R | S | R | R |
| Sulfamethazole | R | S | S | R |
| Ciprofloxacin | S | R | S | S |
| Ranitidine | R | R | R | R |

***R- Resistance S - Sensitive**

**Table4: Biochemical test for identification of bacteria**

| **Morphology test** | **Isolate I** | **Isolate II** |
|---|---|---|
| Gram Staining | + | - |
| Shape | Rod | Rod |
| Endospore staining | - | - |
| Motility | Motile | Non motile |
| **Biochemical Test** | | |
| Indole Test | - | - |
| Methyl red Test | - | - |
| Vogesproskauer Test | - | - |
| Citrate utilisation Test | + | + |
| Catalase Test | - | - |
| Oxidase Test | - | - |
| Triple sugar iron Test | Butt and Slant – yellow colour | Butt and Slant – yellow colour |

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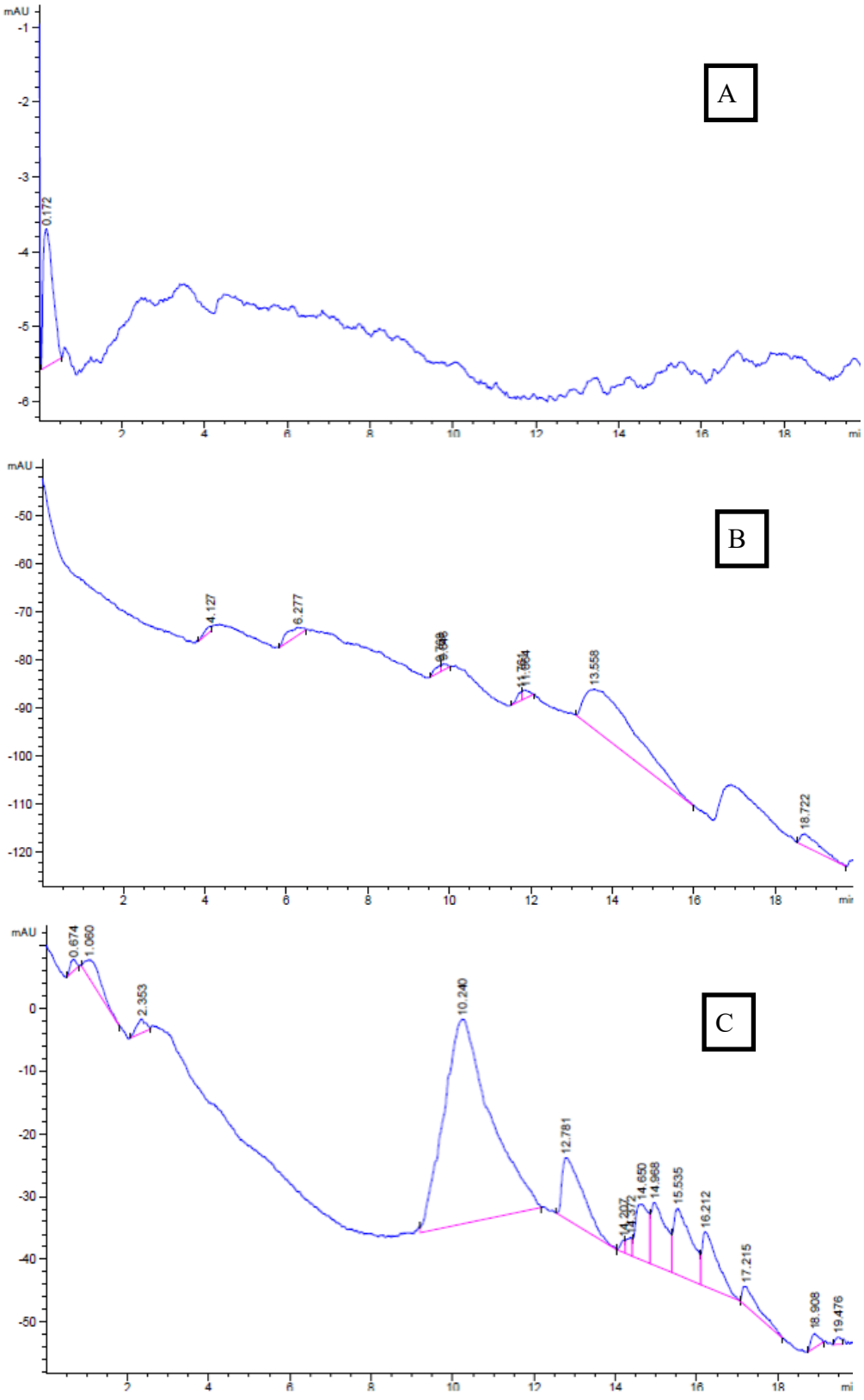


**Figure 1: HPLC analysis for the pharmaceutical compounds: A) Ciprofloxacin B) Ranitidine C) Sulfamethazole**

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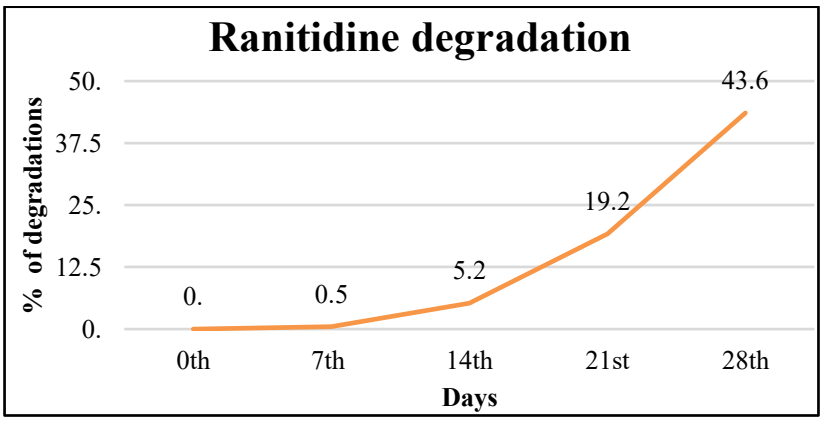


**Figure 2: Degradation of target drug compound by bacterial consortium**

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