Indian Science Technology and Engineering facilities Map

(I-STEM)

Application for

1. **Partial financial support for using R&D facilities in other institutions through the I-STEM web portal to Users from Academic Institutions and Start-ups (Format-1)**

Format for requesting support for accessing and using facilities through the I-STEM web portal.

(i) Details of the Personal Information

a. Name of the Researcher: **Ms. Prajkta Shahaji Sarkale**
b. Name of the Institution: **Shivaji University, Kolhapur**
c. Name of the Department: **Department of Environmental Science**
d. Degree registered for: **Ph.D.**
e. Mob. No.: **9011179657**
f. Email ID: **sprajktaenvsc@gmail.com**

(ii) **Project/Research topic Details**:

a. Project Title/Research Title:

"Evaluation of Antibiotic Residue in Surface Waters of Kolhapur City, It's Potential for Development of Antimicrobial Resistance and Eco-toxicological Impacts".

b. Sub area: **Environmental Science**

c. Area Description:

Antibiotic residue contamination in water can cause a complex impact on the environment, such as the development of antimicrobial resistance. Antibiotic resistance is the ability of organisms to grow in the presence of drugs which are made to inhibit or kill them. Infections associated with antimicrobial-resistant microorganisms become harder to treat, which is a potential health hazard. Antibiotic pollution has posed a major global threat as this antibiotic residue is prevalent in many aquatic bodies. With respect to this scenario proposed work will be carried out to monitor the antibiotic residue of the most frequently used ten selected broad-spectrum antibiotics in the outlets of selected hospitals and their tributaries including river water samples in Kolhapur city.

This work will allow us to know about the current status of antibiotic residue in the water bodies of Kolhapur city. This data will be helpful for policy makers to form rules and regulations related to the wise use of antibiotics.

The data generated in the present work will also be beneficial to prevent or minimize the antibiotic contamination of water bodies by implementing various water treatment techniques at major sources such as hospital wastewater.

Previous studies have suggested that antibiotics contaminate all kind of human food products such as vegetables, livestock, aquatic products and poultry products. So, in the present work, there will be an analysis of plant uptake capacity by growing onion plants in soil spiked with antibiotics. This experiment will be carried out in the laboratory. And all water samples along

with plant extracts after the experiment will be analysed on LC-MS/MS for qualitative and quantitative detection of selected broad-spectrum antibiotics.

Antibiotic residue degradation will be carried out by using advanced oxidation process. Most efficient method will be suggested to hospital waste water treatment plant to prevent the residue in water as well as in food products.

d. Aims & Objective (in Bullets):

- To monitor and analyse the water samples from different Kolhapur city for presence of antibiotic residue.
- To study the eco-toxicological effect by investigating plant uptake capacity of antibiotic residue.
- To study antibiotic susceptibility of clinical isolates by disc diffusion method.
- To suggest low cost and efficient treatment to the hospital wastewater discharge.

e. Duration (MM/YYYY): **6 Months**

f. Project Summary (1000 words):

Antibiotics are medicines used to prevent and treat bacterial infections. India is one of the world's largest consumers of antimicrobials, with easy over-the-counter availability of cheap antibiotics leading to indiscriminate use and misuse (WHO). Antibiotic resistance occurs when bacteria change in response to the use of these medicines. These bacteria may infect humans and animals and the infections they cause are harder to treat than those caused by non-resistant bacteria.

Among the entire pharmaceutical drug that causes contamination of the environment, antibiotics have an important place due to their high use in both veterinary and human medicines. The issue that is created by the antibiotic residue at low concentration in the environment is responsible for the development of antibiotic-resistant microorganisms (Walter et al., 1985). The incidence of development of antimicrobial resistant microorganisms has increased day by day and this increase is due to the excessive use of antibiotics (Kenay, 1983). A number of antibiotics and antimicrobial resistant microorganisms have been reported in the diverse environment such as aquaculture (Zhao et al., 2018), livestock farming (Zhang et al., 2013), hospital wastewater (Varela et al., 2014) and domestic sewage (Parnanen et al., 2019). When hospital wastewater gets mixed with water bodies it can contaminate the water body with antibiotic residue. It is suspected that their occurrence can accelerate resistance spread in the environment (Bengtsson-Palme et al., 2017). Both narrow and broad-spectrum antibiotics are detected globally in various environmental samples (Sarpong and Miller, 2015).

In the proposed research work sampling site will be selected on the basis of major sources of antibiotic residue such as hospital wastewater and associated tributaries. Also to know about the effect of antibiotic residue contaminated water on the plants, a lab experiment will be carried out to study plant uptake

capacity by growing plants of onion in soil spiked with antibiotics. After 3 and 6 weeks plants will be harvested and extracts of water samples, as well as plant samples, will be analyzed for qualitative and quantitative detection of the antibiotic residue using highly sensitive and selective 'Liquid Chromatography tandem mass spectrometry (LC-MS/MS)' techniques. For analysis of antibiotic residue most frequently used ten broad spectrum antibiotics will be used which includes, Chloramphenicol, Tetracycline, Gentamycin, Kenamycin, Neomycin, Vencomycin, Ampicilline, Rifampicine, Nalidixic acid, Streptomycin.

Monitoring antibiotic residue concentration in different water sources in Kolhapur city and antibiotic uptake capacity of plants will be helpful in providing a realistic picture of the status and trends related to the use of antibiotics.

The data generated in this research will go a long way in creating awareness among regulators, producers and consumers and will also be beneficial in developing strategies for judicious use of antibiotics. This work is intended to highlight some key risks associated with health. This work will shed light on the current scenario and that will help to create awareness and to find out some solutions to resolve or minimize the negative impacts associated with antibiotic residue. The proposed study aims to provide comprehensive monitoring data on antibiotic residue in surface water. It will highlight major sources of antibiotic residue in surface water. Results will be helpful to minimize the concentration of antibiotics from sources by implementing suitable treatments and ultimately help to reduce associated consequences such as widespread antimicrobial resistance and the development of allergic conditions in the population.

g. **Technical Details**

a. TRL: 3

b. Methodology:

Water sample collection will be done in Kolhapur city from multispecialty hospital outlets and their tributaries. Antibiotic residue analysis for selected board spectrum antibiotics will be performed using highly sensitive and selective 'Liquid Chromatography tandem mass spectrometry (LC-MS/MS)' techniques. For investigating plant uptake capacity for antibiotic residue standard antibiotic concentrations will be supplemented to plant and after two weeks plant leaves will be analyzed for antibiotics residue concentration by using LC-MS/MS. For degradation of selected antibiotics advanced oxidation method will be used and most efficient and low cost method will be determined.

For antibiotic susceptibility study clinical isolates will be used and by disc diffusion method pattern of antibiotic resistant bacteria will be evaluated.

c. Design & development:

Water samples will be collected from the selected sampling sites.

Initial water parameters will be analyzed as per standard methods in the laboratory.

Extraction of collected water samples will be done by using solid phase extraction.

The extracted samples will be analyzed for qualitative and quantitative detection for antibiotic residue of ten selected broad-spectrum antibiotics.
For investigating plant uptake capacity, in the experiment green onion (four plants per pot will be transplanted in soil spiked with selected antibiotics. After transplanting, two onion plants from each pot after 3 and 6 weeks will be harvested and extracted sample will be analyzed for antibiotic residue detection using LC-MS/MS.
Degradation of antibiotic will be examined in lab by using advanced oxidation process.

d. Facilities and Instrumentation required:
'Liquid Chromatography tandem mass spectrometry (LC-MS/MS)'
Solid phase extraction assembly (Cartridges)
COD digester

e. Consumables (Scientific materials):
Chemicals and lab aids required are listed below (Quotation attached)

Sr. No.	**Chemicals**
1	Amphotericin B
2	Ampicillin sodium salt
3	Kanamycin monosulphate
4	Gentamicin sulphate
5	Rifampicin
6	Streptomycin sulphate
7	Tetracycline hydrochloride
8	Nalidixic acid, Free acid
9	Neomycin sulphate
10	Vancomycin hydrochloride
11	Chloramphenicol
12	Hydrogen peroxide
13	Zinc Oxide
14	Ferrous sulphate heptahydrate
15	PET Bottle, sterile, 1000 ml
16	PET Bootle sterile, 125 ml
17	Azithromycin
18	Ciprofloxacin
19	amoxyclav
20	Ampicillin
21	Ciprofloxacin
22	Gentamicin
23	Kenamycin
24	Nalidixic acid

25	Tetracycline
26	Streptomycin
27	Vancomycin
28	Neomycin
29	Rifampicin

h. **Expected output and outcome**

The proposed study aims to provide comprehensive monitoring data on antibiotic residue in surface water. It will highlight and monitor major sources of antibiotic residue. Results will be helpful to minimize the concentration of antibiotics from sources by implementing suitable treatments and ultimately help to reduce associated consequences such as widespread antimicrobial resistance and allergic condition development in the population. Treatment method evaluated during research work will be helpful for treating antibiotic residue contaminated water from major sources such as hospital waste water treatment plants for removal of antibiotic residue.

i. **Industry/Institute Collaboration** **If, yes**

(i) Name of the Collaborating Industry/Institute
(ii) Collaboration Details
(iii) Additional Details if any

j. **Funding required from I-STEM (with justification:**

Details of Requirements & Mode of the Engagement: (Attach the quotation for the consumables)

(i) Short term Engagement:

a. Usage of testing/characterization facilities across the country (Characterization Mode)

i. Testing Charges: Rs. 1,44,000/- (LC-MS/MS analysis charges including extraction 3600/sample X 40 samples)
Sample testing will be carried out at ICAR National Research Centre for Grapes, Pune.
ii. Consumable charges: Rs. 50,000/- (Approximate)
Rs. 39,350/- +18% GST (Chemicals and lab aids quotation attached)
iii. Travel Cost (Boarding and Lodging, if any): Rs. 15,000/- (traveling from Kolhapur to Pune and return to Kolhapur + travelling for collection of samples in Kolhapur + Lodging if required at the time of sample analysis s in Pune)

(Round-trip to the Host Institution by 2nd class (Non-AC) railway via the nearest route)

Details of User's Bank Account:

(Copy of the passbook is attached.)

Beneficiary Name (User's name) : **Prajkta Shahaji Sarkale** PAN: **KFVPS3947Q**

Bank Account No.: **35803848642** Name of the Branch: **State Bank of India, Umbraj**

IFSC Code: **SBIN0011132**