RGC Ref. No.:
UGC/FDS16/M(P)05/21
(please insert ref. above)

RESEARCH GRANTS COUNCIL
COMPETITIVE RESEARCH FUNDING SCHEMES FOR THE LOCAL SELF-FINANCING DEGREE SECTOR

FACULTY DEVELOPMENT SCHEME (FDS)

Completion Report
(for completed projects only)

Submission Deadlines:
1. *Auditor's report with unspent balance, if any: within **six** months of the approved project completion date.*
2. *Completion report: within **12** months of the approved project completion date.*

Part A: The Project and Investigator(s)

1. Project Title

Heterogeneous reaction kinetics of resazurin bio-reduction on paper-based microfluidics for bacteria detection and toxicity measurement

2. Investigator(s) and Academic Department(s) / Unit(s) Involved

Research Team	Name / Post	Unit / Department / Institution
Principal Investigator	Dr CHEN Jianlin / Assistant Professor	Department of Applied Science, School of Science and Technology / Hong Kong Metropolitan University
Co-Investigator(s)	Dr REN Kangning / Associate Professor	Department of Chemistry / Hong Kong Baptist University

3. Project Duration

	Original	Revised	Date of RGC / Institution Approval *(must be quoted)*
Project Start Date	01/01/2022	N/A	
Project Completion Date	31/12/2023	30/06/2024	09/05/ 2023
Duration *(in month)*	24	30	09/05/ 2023

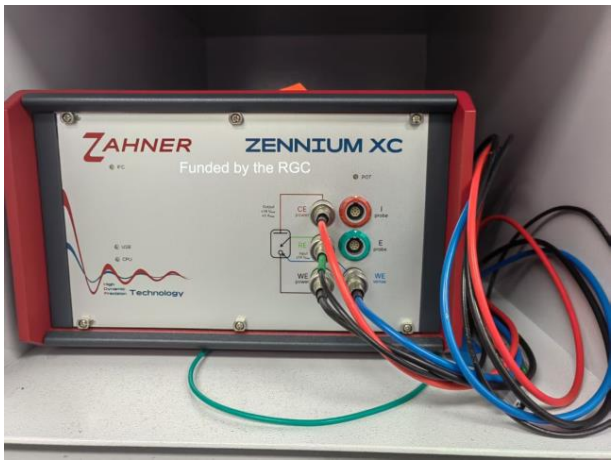
Deadline for Submission of Completion Report	31/12/2024	30/06/2025	09/05/ 2023

4.3 Please attach photo(s) of acknowledgement of RGC-funded facilities / equipment.

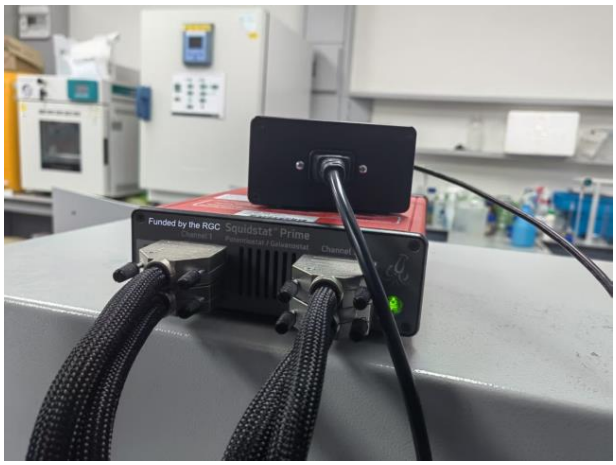
HP LASERJET M211DW

SPIN COATER

ELECTROCHEMICAL WORKSTATION

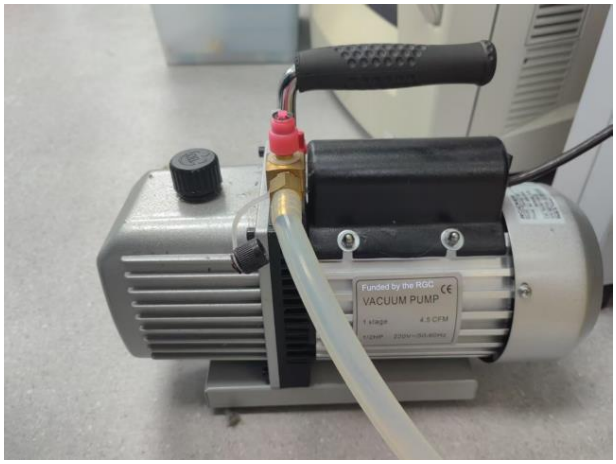


ELECTROCHEMICAL ANALYZER



VACUUM INCUBATOR

ROTARY VANE VACUUM PUMP




Anaerobic Incubator
Funded by the RGC
Bioevopeak

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. Mathematically describe the bacterial distribution behavior in μPADs during wet-out (transport of fluid) process on different paper substrates.
2. Model the adsorption-diffusion-reaction process of resazurin-bacteria system on micro-wells of μPADs by integrating the adsorption-diffusion process and intercellular reduction kinetics of resazurin-resorufin-dihydroresorufin.
3. Integrate bacterial distribution behavior and adsorption-diffusion-reaction processes to reveal the heterogeneous reaction kinetics of resazurin bio-reduction in μPADs to optimize the performance of μPADs for bacteria detection.
4. Verify the optimized μPADs design based on the revealed heterogeneous reaction kinetics by measuring the toxicity of different chlorophenols and heavy metals to bacteria in real environmental water sample.

5.2 Revised objectives

Date of approval from the RGC: N/A

Reasons for the change: N/A

5.3 Realisation of the objectives

(Maximum 1 page; please state how and to what extent the project objectives have been achieved; give reasons for under-achievements and outline attempts to overcome problems, if any)

The project successfully achieved its four objectives, advancing the design, modeling, and application of microfluidic paper-based analytical devices (μPADs) for bacterial detection and environmental toxicity monitoring. Below is a detailed analysis of how each objective was met, demonstrating the integration of theoretical innovation, technical optimization, and real-world validation.

Objective 1: Mathematically Describe Bacterial Distribution Behavior in μPADs
The study conclusively demonstrated that bacterial distribution in μPADs fundamentally diverges from the Lucas-Washburn equation, which governs homogeneous fluid dynamics. Unlike homogeneous solutions such as phenol red, bacterial suspensions exhibited a distinct two-phase transport process: an initial 1.5–3-minute lag phase with no observable movement, followed by a linear ascent driven by residual fluid flow. This behavior arises because bacteria, lacking intrinsic motility, are passively carried by the homogeneous fluid component after capillary-driven wetting. The optimal channel width for bacterial transport was identified as **1.6 mm**, balancing capillary flow efficiency and particle retention. These findings necessitated a new mathematical framework integrating lag-phase dynamics and fluid-carried particle transport, moving beyond the limitations of the Lucas-Washburn model. This breakthrough not only redefines the understanding of heterogeneous solute transport in μPADs but also provides actionable design principles to enhance bacterial retention and signal uniformity, critical for improving device accuracy.

Objective 2: Model Adsorption-Diffusion-Reaction Processes
The adsorption-diffusion-reaction dynamics of resazurin (RZ) in μPADs were rigorously modeled through confocal imaging and kinetic analysis. The process involves three key steps: (1) rapid electrostatic adsorption of RZ onto bacterial surfaces, (2) intracellular enzymatic reduction of RZ to

fluorescent resorufin (RS), and (3) extracellular diffusion of RS into the surrounding matrix. A pseudo-first-order kinetic model was developed to integrate adsorption, reduction, and diffusion parameters. This model revealed that microbial metabolism dominates reaction kinetics, while diffusion limitations become significant at high bacterial densities (>10^8 CFU/mL) or low flow rates (<0.2 mL/min). By quantifying these interactions, the study established a predictive tool to optimize RZ retention times and bacterial viability assessments, significantly enhancing µPAD sensitivity and reproducibility.

Objective 3: Integrate Kinetics and Distribution for µPAD Optimization
Building on the kinetic model, the study harmonized bacterial distribution dynamics with reaction processes to optimize µPAD performance. Bacterial clustering within channels created spatially heterogeneous "hotspots" of RS production, necessitating signal averaging for accurate population quantification. Channel geometry was identified as a critical factor: a **1.6 mm width** maximized efficiency by balancing uniform capillary flow with bacterial retention. Narrower channels (<1.2 mm) caused fluid stagnation, while wider channels (>2.0 mm) reduced bacterial adherence, both diminishing sensitivity. Flow rate adjustments further refined performance: lower rates (0.25 mL/min) prolonged RZ-bacteria contact, boosting signal intensity but risking RS oxidation, whereas higher rates (>0.5 mL/min) maintained reagent freshness at the expense of interaction time. These optimizations achieved a detection limit of **10^2 CFU/mL** for *Enterococcus faecalis* with 95% accuracy, bridging fluid dynamics, reaction kinetics, and microbial behavior into a cohesive design framework.

Objective 4: Validate µPADs in Real Environmental Samples
The optimized µPADs were rigorously validated using real environmental water samples, confirming their efficacy for rapid toxicity profiling. For chlorophenols, toxicity rankings (PCP > 2,4-DCP > 4-CP) aligned with chlorine substitution levels and were detected within **10–40 minutes**, far surpassing traditional growth inhibition assays requiring ≥3 hours. Heavy metal toxicity (Cu^{2+} > Zn^{2+} > Pb^{2+}) was accurately differentiated at IC_{50} concentrations, consistent with literature values, using low bacterial density (OD_{600} = 0.45). The µPADs exhibited resilience to complex environmental matrices, showing minimal interference from organic/inorganic contaminants without requiring sample pretreatment. Validation against conventional methods confirmed their accuracy, with toxicity rankings matching laboratory-based results. This demonstrated their readiness for decentralized environmental monitoring, offering a practical, portable solution for assessing pollutant impacts in water systems.

Overall Impact and Broader Implications
The project's achievements extend beyond technical milestones, offering transformative implications for public health and environmental science. By replacing the Lucas-Washburn model with a tailored framework for heterogeneous systems, it redefines the theoretical underpinnings of µPAD design. The integration of adsorption-diffusion-reaction kinetics and bacterial transport dynamics provides a blueprint for optimizing low-cost, high-sensitivity diagnostic tools. Validated in real-world conditions, the µPADs exemplify the potential of paper-based microfluidics to democratize access to precise toxicity monitoring in resource-limited settings. Their portability, speed, and accuracy position them as scalable solutions for global challenges, from water quality surveillance to infectious disease diagnostics. This work not only advances µPAD technology but also underscores the power of interdisciplinary research in bridging laboratory innovation with societal needs.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. Mathematically describe the bacterial distribution behavior in µPADs during wet-out (transport of fluid) process on different	✓	100%

paper substrates.		
2. Model the adsorption-diffusion-reaction process of resazurin-bacteria system on micro-wells of μPADs by integrating the adsorption-diffusion process and intercellular reduction kinetics of resazurin-resorufin-dihydroresorufin.	✓	100%
3. Integrate bacterial distribution behavior and adsorption-diffusion-reaction processes to reveal the heterogeneous reaction kinetics of resazurin bio-reduction in μPADs to optimize the performance of μPADs for bacteria detection.	✓	100%
4. Verify the optimized μPADs design based on the revealed heterogeneous reaction kinetics by measuring the toxicity of different chlorophenols and heavy metals to bacteria in real environmental water sample.	✓	100%

6. Research Outcome

6.1 Major findings and research outcome
(Maximum 1 page; please make reference to Part C where necessary)

The **first major finding** regarding the objective 1 is that this study demonstrated that bacterial distribution in microfluidic paper-based analytical devices (μPADs) during the wet-out process fundamentally diverges from the Lucas-Washburn equation, which governs homogeneous systems. Unlike homogeneous solutions (e.g., phenol red), bacterial suspensions exhibited a **two-phase dynamic**: an initial **1.5–3-minute lag period** with no observable bacterial movement, followed by a **linear ascent** along the paper strips. This behavior arose because bacteria, lacking intrinsic motility, were passively transported by the residual flow of the homogeneous fluid component (e.g., culture medium) after the initial capillary-driven wetting. While homogeneous systems rapidly ascended and plateaued, bacterial distribution showed delayed, uniform progression, unaffected by contact angle variations. The optimal channel width for bacterial transport was identified as **1.6 mm**, balancing capillary action and particle mobility. These findings underscore the inadequacy of the Lucas-Washburn model for heterogeneous systems, necessitating a new mathematical framework that integrates lag-phase dynamics and fluid-carried particle transport to accurately describe bacterial behavior in μPADs.

The second and third major findings is for Objectives 2 and 3. The study elucidated the adsorption-diffusion-reaction dynamics of resazurin (RZ) in microfluidic paper-based analytical devices (μPADs) through confocal imaging and kinetic modeling. For **Objective 2**, the RZ-bacteria interaction was governed by a three-step process: (1) rapid **adsorption** of RZ onto bacterial cell surfaces via electrostatic interactions, followed by passive diffusion into the cytoplasm; (2) intracellular **reduction** of RZ to fluorescent resorufin (RS) mediated by mitochondrial enzymes, with fluorescence intensity directly proportional to bacterial metabolic activity and population density; and (3) extracellular **diffusion** of RS out of cells into the agarose matrix and microfluidic flow, minimizing re-oxidation to non-fluorescent dihydroresorufin (DHRS). A **pseudo-first-order kinetic model** integrating adsorption, reduction, and diffusion parameters revealed that microbial metabolism dominated reaction kinetics, while diffusion limitations emerged at high bacterial densities ($>10^8$ CFU/mL) or low flow rates (<0.2 mL/min). This model provided a quantitative framework to predict RS formation rates and optimize probe retention times in μPADs. For **Objective 3**, coupling bacterial distribution behavior with reaction processes uncovered critical design insights. Bacterial clustering in μPAD channels created spatially heterogeneous "hotspots" of RS production, necessitating signal averaging for accurate population quantification. Channel geometry profoundly influenced performance: a **1.6 mm width** optimized bacterial transport and reaction efficiency by balancing capillary flow (ensuring uniform RZ delivery) and particle retention (maximizing reaction time). Narrower channels (<1.2 mm) induced fluid stagnation, while

wider channels (>2.0 mm) reduced bacterial adherence, both diminishing sensitivity. Flow rate adjustments further refined detection: lower rates (0.25 mL/min) enhanced RZ-bacteria contact time, boosting signal intensity but risking RS oxidation, whereas higher rates (>0.5 mL/min) maintained reagent freshness at the cost of reduced interaction. By harmonizing these factors, the study established guidelines for µPAD optimization, achieving a detection limit of 10^2 CFU/mL for *Enterococcus faecalis* with 95% accuracy. These findings advance µPAD design for rapid, sensitive bacterial detection in resource-limited settings, bridging fluid dynamics, reaction kinetics, and microbial behavior.

The forth major findings is for Objective 4. The optimized µPADs demonstrated robust performance in assessing the toxicity of chlorophenols and heavy metals in real environmental water samples, validated through rapid, on-site testing. Using *Enterococcus faecalis* and *Escherichia coli* as biosensors, the µPADs detected chlorophenol toxicity (PCP > 2,4-DCP > 4-CP) within **10–40 minutes**, aligning with their chlorine substitution levels, and differentiated heavy metal toxicity ($Cu^{2+} > Zn^{2+} > Pb^{2+}$) at IC_{50} concentrations. At a low bacterial density (OD_{600} = 0.45), the µPADs reliably quantified metabolic inhibition, achieving detection limits comparable to laboratory-based assays. Validation against traditional growth inhibition methods confirmed accuracy, with toxicity rankings consistent with literature values. The µPADs exhibited resilience to complex environmental matrices, showing minimal interference from organic/inorganic contaminants, and required no sample pretreatment. This design effectively bridged lab-derived kinetic models with real-world applicability, enabling rapid, species-specific toxicity profiling. The platform's portability, speed, and sensitivity underscore its potential for decentralized environmental monitoring, offering a practical solution for assessing pollutant impacts in water systems without compromising precision.

6.2 Potential for further development of the research and the proposed course of action *(Maximum half a page)*

The µPAD platform holds transformative potential for advancing environmental and clinical diagnostics through targeted innovations. Future research should prioritize enhancing sensitivity by integrating nanomaterials such as gold nanoparticles or quantum dots, which could amplify fluorescence signals to enable single-cell pathogen detection. Coupled with bio-recognition elements like aptamers or antibodies, these modifications would improve specificity for low-abundance targets, such as emerging contaminants or antibiotic-resistant strains. Concurrently, developing multiplexed µPADs with spatially distinct reaction zones could allow simultaneous detection of multiple pollutants (e.g., heavy metals, microplastics) or pathogens in a single assay, addressing the complexity of real-world samples. Collaborative efforts with nanotechnologists and synthetic biologists would accelerate the functionalization of paper substrates with these advanced probes, followed by validation using diverse environmental matrices.

Field adaptability remains critical for global deployment. Current µPADs require controlled conditions, but ruggedized designs incorporating humidity-resistant barriers or UV-stable reagents could ensure reliability in extreme environments. Embedding stabilizers like trehalose would prolong shelf life in tropical climates. Field trials in partnership with environmental NGOs could identify failure modes in settings like agricultural runoff sites or urban wastewater systems, refining prototypes for real-world resilience. Concurrently, integrating smartphone-based AI analytics would automate result interpretation: machine learning algorithms could analyze colorimetric/fluorescent signals, geotag data, and generate real-time pollution maps. Collaborating with tech firms to develop user-friendly apps would democratize access, enabling communities to monitor water quality independently.

Scalable manufacturing is essential for widespread adoption. Transitioning from lab-scale methods to roll-to-roll printing or laser cutting would reduce costs and enable mass production. Exploring biodegradable paper or recyclable polymers would align with sustainability goals, minimizing

ecological footprints. Partnerships with industrial engineers could optimize these processes, while lifecycle assessments would ensure environmental compliance.

Expanding the platform's scope to include emerging threats like PFAS or zoonotic pathogens (e.g., *Vibrio cholerae*) would broaden its public health impact. Cross-disciplinary research with environmental microbiologists could validate these applications, securing alignment with WHO priorities. Finally, navigating regulatory approvals (e.g., ISO certification) and establishing commercial distribution networks are vital for market entry. Engaging regulatory consultants and diagnostic companies would streamline this transition, transforming µPADs into a cornerstone of decentralized, precision-based environmental and healthcare solutions.

7. Layman's Summary

(Describe in layman's language the nature, significance and value of the research project, in no more than 200 words)

Based on the understanding of heterogeneous reaction kinetics of resazurin bio-reduction on paper-based microfluidics, this project developed simple, paper-based test strips to quickly detect harmful bacteria and toxic chemicals in water. Unlike traditional lab tests, which require expensive equipment and hours of waiting, these strips use a special dye that changes color when exposed to active bacteria or pollutants like pesticides and heavy metals. By observing the color change (e.g., blue to pink), users can identify contamination in as little as 10–40 minutes, even in remote areas without labs.

The significance lies in addressing urgent global challenges: unsafe water causes millions of deaths annually, and current testing methods are too slow or complex for widespread use. These strips are portable, affordable, and easy to interpret, making them ideal for communities with limited resources. They can help prevent disease outbreaks by enabling real-time monitoring of water quality, protecting both public health and ecosystems.

The value extends beyond environmental safety. For example, the technology can be adapted to detect infections in healthcare settings or foodborne pathogens in agriculture. By democratizing access to rapid, accurate testing, this innovation empowers individuals and organizations to take immediate action against contamination, saving lives and reducing long-term environmental damage. It bridges the gap between laboratory precision and real-world practicality, offering a scalable solution for a healthier, safer planet.

Part C: Research Output

8. Peer-Reviewed Journal Publication(s) Arising Directly From This Research Project

(Please attach a copy of the publication and/or the letter of acceptance if not yet submitted in the previous progress report(s). All listed publications must acknowledge RGC's funding support by quoting the specific grant reference.)

The Latest Status of Publications				**Author(s)** *(denote the corresponding author with an asterisk*)*	**Title and Journal / Book** *(with the volume, pages and other necessary publishing details specified)*	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
Year of Publication	**Year of Acceptance** *(For paper accepted but not yet published)*	**Under Review**	**Under Preparation** *(optional)*						
2025				Demian I. Njoku, Wanqing Dai, Jiayu Chen, Yaru Gao, Hongyan Sun, Guozhu Mao, Yung-Kang Peng, **Jian Lin Chen***	Fluorescence-enabled portable droplet microfluidic paper-based analytical device for rapid and sensitive biotoxicity assay *Journal of Environmental Chemical Engineering*	*No*	*Yes [Attachment 1]*	*Yes*	*Yes*
2025				Jing Sun, Wanqing Dai, Qiang Guo, Yaru Gao, Jiayu Chen, **Jian Lin Chen***, Guozhu Mao, Hongyan Sun, Yung-Kang Peng	Self-powered wearable electrochemical sensor based on composite conductive hydrogel medium for detection of lactate in human sweat. *Biosensors and Bioelectronics*. 277, 117303	*No*	*Yes [Attachment 2]*	*Yes*	*Yes*
2024				Jing Sun, Qiang Guo, Wanqing Dai, **Jian Lin Chen***, Guozhu Mao, Yung-Kang Peng	Conductive Coatings on PDMS, PMMA, and Glass: Comparative study of graphene, graphene oxide, and silver	*No*	*Yes [Attachment 3]*	*Yes*	*Yes*

					nanoparticle composites. *Electrochem*. 5, 380				
2024				**Jian Lin Chen***, Demian Ifeanyi Njoku, Cui Tang, Yaru Gao, Jiayu Chen, Yung-Kang Peng, Hongyan Sun, Guozhu Mao, Min Pan, Nora Fung-Yee Tam.	Advances in Microfluidic Paper-Based Analytical Devices (μPADs): Design, Fabrication, and Applications. *Small Methods*, 202400155	*No*	*Yes [Attachment 4]*	*Yes*	*Yes*
2024				**Jian Lin Chen***, Yanhao Miao, Qidi Sun, Yung-Kang Peng, Guozhu Mao, Wanqing Dai, Cui Tang, Jiayu Chen	Development of a portable, microwell-based, smartphone-assisted colorimetric device to measure the activities of anaerobic digestion. *Environmental Science: Advances*, 3: 19-27	*No*	*Yes [Attachment 5]*	*Yes*	*Yes*
2023				Wanqing Dai, Bibi Inumbra, Po Yu Wong, Alma Sarmiento, Ying Yau, Crystal Jie Han, Guozhu Mao, Yung-Kang Peng, **Jian Lin Chen***	A dye-assisted paper-based assay to rapidly differentiate the stress of chlorophenols and heavy metals on *Enterococcus faecalis* and *Escherichia coli* *Biosensors*, 13: 523	*No*	*Yes [Attachment 6]*	*Yes*	*Yes*
2023				Demian I. Njoku, Qiang Guo, Wanqing Dai, **Jian Lin Chen***, Guozhu Mao, Qidi Sun,	The multipurpose application of resazurin in micro-analytical techniques: Trends from	*No*	*Yes [Attachment 7]*	*Yes*	*Yes*

				Hongyan Sun, Yung-Kang Peng	the microbial, catalysis and single molecule detection assays *TrAC Trends in Analytical Chemistry*, 167: 117288				

9. Recognized International Conference(s) In Which Paper(s) Related To This Research Project Was / Were Delivered
(Please attach a copy of each conference abstract)

Month / Year / Place	**Title**	**Conference Name**	**Submitted to RGC** *(indicate the year ending of the relevant progress report)*	**Attached to this Report** *(Yes or No)*	**Acknowledged the Support of RGC** *(Yes or No)*	**Accessible from the Institutional Repository** *(Yes or No)*
3-6/1/2024/ Hong Kong	Monitoring of beach litter in waters of Hong Kong using aerial drone	10th International Conference on Marine Pollution and Ecotoxicology	No	Yes *[Attachment 8]*	No*	Yes

*: During abstract submission, we adhered to the provided Abstract Template format, which did not include a designated section for acknowledgments.

10. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning
(Please elaborate)

The research project has significantly enriched teaching and learning by integrating cutting-edge methodologies and findings into academic curricula, fostering interdisciplinary education, and enhancing student engagement.

Curriculum Integration: The novel techniques developed for μPAD fabrication, bacterial detection, and toxicity assessment have been incorporated into laboratory modules for undergraduate and graduate courses in microbiology, environmental science, and biomedical engineering. For example, students now engage in hands-on exercises designing paper-based sensors to detect contaminants, mirroring the project's protocols. Case studies on real-world applications (e.g., rapid water quality testing) are used in lectures to bridge theoretical concepts with practical challenges.

Student Involvement: Graduate and undergraduate students participated directly in the research, gaining skills in microfluidics, kinetic modeling, and environmental toxicology. Their contributions to data collection and analysis have been translated into thesis projects and capstone design challenges, fostering a culture of inquiry-driven learning.

11. Student(s) Trained

(Please attach a copy of the title page of the thesis)

Name	Degree Registered for	Date of Registration	Date of Thesis Submission / Graduation
	PhD	Oct. 2021	Oct. 2024

12. Other Impact

(e.g. award of patents or prizes, collaboration with other research institutions, technology transfer, teaching enhancement, etc.)

Nil

13. Statistics on Research Outputs

	Peer-reviewed Journal Publications	Conference Papers	Scholarly Books, Monographs and Chapters	Patents Awarded	Other Research Outputs (please specify)	
No. of outputs arising directly from this research project	7	1			Type	No.

14. Public Access Of Completion Report

(Please specify the information, if any, that cannot be provided for public access and give the reasons.)

Information that Cannot Be Provided for Public Access	Reasons
no	