RGC Ref. No.:
UGC/FDS25(16)/M01/20
(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

The fate and effects of PPCPs in soil-plant systems and their metabolization by plants with potential use in phytotreatment/remediation applications

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

Research Team	**Name / Post**	**Unit / Department / Institution**
Principal Investigator	Dr PAN Min / Senior Lecturer	Department of Applied Science, School of Science and Technology, Hong Kong Metropolitan University
Co-Investigator(s)	Prof. Wong, Jonathan Woon Chung/ Professor and Head of Department	Department of Biology, Hong Kong Baptist University
Co-Investigator(s)	Dr Zhang Hao/ Associate Professor	Faculty of Design and Environment, Technological and Higher Education Institute of Hong Kong

3. Project Duration

	Original	**Revised**	**Date of RGC / Institution Approval** *(must be quoted)*
Project Start Date	01/01/2021	NA	NA
Project Completion Date	30/06/2023	31/12/2023	14/03/2023
Duration *(in month)*	30	36	14/03/2023
Deadline for Submission of Completion Report	30/06/2024	31/12/2024	14/03/2023

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

Part B: The Final Report

5. Project Objectives

5.1 Objectives as per original application

1. To assess the distribution and degradation of PPCPs in bulk soil and in pore water to gain more insight into the bioavailable fractions of PPCPs in soil for plant root uptake;
2. To investigate the effects and toxicity of PPCPs on microbial and earthworm responses in soil;
3. To study the metabolization mechanisms of PPCPs by plants;
4. To evaluate the phytotreatment and remediation ability of target plants on PPCPs;
5. To obtain useful information on the risk quotients of target plants to PPCPs from the reuse of treated wastewater and biosolids in soils.

5.2 Revised objectives

Date of approval from the RGC: NA

Reasons for the change:

1.

2.

3. ....

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)

Objective 1: we investigated the potential phytoremediation pathways for these two drugs in plant systems by tracking and quantifying the fate of the parent compounds and their metabolites in *Arabidopsis thaliana* using cell and seedling cultures. Results indicated significant differences in the dissipation of DLF according to the treatment and time interaction within the cell cultures. Viable plant cells showed complete dissipation of DLF from an initial concentration of 2,758 ng/mL in 96 h, whereas non-viable cells and blank solutions remained stable. The dissipation of SMX was comparable across viable, non-viable, and blanks, showing a minor decrease from 842 to 799 ng/mL over 120 hours following the treatment of viable cells.
Objective 2: we investigated the effects and toxicity of PPCPs on microbial and

earthworm responses in soil; Earthworm assessments were based on the Earthworm Sub-chronic Toxicity Test (OCSPP 850.3100). Microbial impacts were assessed using respiration and reverse transcriptase-quantitative PCR (mRNA) analyses of nutrient (N and P) cycling genes as toxicity markers.

Objective 3: we studied the metabolization mechanisms of PPCPs by plants; DLF metabolites including 4'-hydroxy-diclofenac, 5-hydroxy-diclofenac, acyl-glutamatyl-diclofenac, 1-(2,6-dichlorophenyl)-5-hydroxy-2-indolinone, 5-sulfooxy-diclofenac, 5-glucopyranosyloxy-diclofenac, 1-(2,6-dichloro-4-hydroxyphenyl)-2-indolinone, and 4'-glucopyranosyloxy-diclofenac were recognized, likely formed through acylation, glutamyl conjugation, hydroxylation, dehydration, cyclization, sulfonation, and glucosidation. While for SMX, metabolites including sulfamethoxazole-glucuronide, nitroso-sulfamethoxazole, N_4-acetylsulfamethoxazole, and N_4-acetyl-5-OH-sulfamethoxazole were identified, potentially produced through glucuronidation, nitrosation, acetylation, and hydroxylation. Phase I metabolite concentrations of DLF and SMX peaked earlier than those of phase II metabolites.

Objective 4: we evaluated the phytotreatment and remediation ability of target plants on PPCPs; Hydroponic *A. thaliana* demonstrated comparable efficiencies in the phytoremediation of DLF and SMX, with concentrations varying from 1 mg/L to 10 mg/L. Detectable levels of both parent compounds and their metabolites confirmed successful absorption and metabolism within the plant system. This study provides valuable insights into the potential of phytoremediation as a sustainable approach for reducing the environmental toxicity of DLF and SMX and suggests comparable metabolic efficiency. These findings contribute to the growing body of knowledge on phytoremediation and its application in addressing pollution from pharmaceuticals and personal care products.

Objective 5: we obtained useful information on the risk quotients of target plants to PPCPs from the reuse of treated wastewater and biosolids in soils. These findings contribute to the growing body of knowledge on phytoremediation and its application in addressing pollution from pharmaceuticals and personal care products.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. To assess the distribution and degradation of PPCPs in bulk soil and in pore water to gain more insight into the bioavailable fractions of PPCPs in soil for plant root uptake;	√	100%
2. To investigate the effects and toxicity of PPCPs on microbial and earthworm responses in soil;	√	100%
3. To study the metabolization mechanisms of PPCPs by plants;	√	100%
4. To evaluate the phytotreatment and remediation ability of target plants on PPCPs;	√	100%
5. To obtain useful information on the risk quotients of target plants to PPCPs from the reuse of treated wastewater and biosolids in soils.	√	100%

6. Research Outcome

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

(1) In study 1, we have investigated the bioconcentration of SMX and DLF in bulk soil and pore water to gain more insight into the bioavailable fractions of SMX and DLF in soil for *Arabidopsis thaliana* and carrot root uptake. We found the degradation pathways of target SMX and DLF and analyzed the precursor compounds and their degraded products in soil-plant systems. Through this study, we have determined the bioconcentration and degradation pathways of SMX and DLF in soils. Some related data have been published on the international journal, please refer to Part C.

(2) In study 2, we have evaluated the effects of SMX and DLF on microbial and earthworm responses in soil. We found that high concentrations of SMX and DLF exhibited toxicity and bioaccumulation behavior in soil microbes and earthworms. Respiration and RT-qPCR analyses have been performed for the data analysis. The bioaccessibility and toxicity, accumulation and degradation of SMX and DLF have been assessed and calculated and correlated with microbial and earthworm responses. The related data will be published in the international journal soon.

(3) In study 3, we have assessed SMX and DLF accumulation and metabolization mechanisms by *Arabidopsis thaliana* and carrot, and also determined the corresponding phytotreatment or remediation methods. Hydroponic cultivation for *Arabidopsis thaliana* and carrot, five different trays represent different treatments (control groups, SMX 1 μg/L, SMX 10 μg/L, DLF 1 μg/L and DLF 10 μg/L) have been conducted. Results indicated significant differences in the dissipation of DLF according to the treatment and time interaction within the cell cultures. The results revealed significant interactions between treatment and incubation time, indicating the dissipation of the parent compounds over a 120 h incubation period. Viable cell cultures showed a significant decrease in DLF concentration, while viable cell cultures exhibited only a slight decrease in SMX concentration. Multiple metabolites were identified for both DLF and SMX, and various metabolic processes are involved in their formation. For DLF, metabolites such as acyl-glutamate-diclofenac, 5-hydroxyl-diclofenac, and 5-sulfooxy-diclofenac were observed, indicating hydroxylation, acylation, glutamyl conjugation, and other processes. SMX produced metabolites, including sulfamethoxazole-glucuronide, nitroso-sulfamethoxazole, and N_4-acetylsulfamethoxazole, suggesting that glucuronidation, nitrosation, acetylation, and hydroxylation are important metabolic processes. In *A. thaliana* seedlings treated with different concentrations of DLF and SMX over a 28 d incubation period, a proportional difference in the concentration of the parent compound was still evident. The concentrations of phase I and some phase II metabolites in the seedlings were proportional to the initial concentration of the parent compound. However, some phase II metabolites were not detected, potentially due to their low concentration or further metabolism. Overall, this study provides valuable insights into the potential of phytoremediation as a sustainable approach for reducing the environmental toxicity of pharmaceuticals, specifically within the concentration range of 1-10 mg/L. The consistent metabolism of DLF and SMX within this range suggested comparable metabolic efficiency. These findings contribute to the growing body of knowledge on phytoremediation and its application in addressing pollution from PPCPs.

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

The detection and analysis methods developed for SMX and DLF in this study can be used in commercial labs or attract R&D investment, which can enhance the research capacity, knowledge and skills of private and third sector organizations. Professionals and practitioners of the agricultural industry can yield useful information on the risk quotients of SMX and DLF to target plants, through the reuse of treated wastewater and biosolids in agriculture. This information will improve long-term environmental sustainability, soil protection, and impact. Policymakers of the

agricultural field can know more about the fate, degradation pathways and bioaccumulation of SMX and DLF in soil-plant systems, as well as food safety. The information helps them to devise solutions to improve social welfare, social cohesion, and national security.

Layman's Summary

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

Pharmaceuticals and personal care products (PPCPs) like sulfamethoxazole (SMX) and diclofenac (DLF) often end up in the environment through wastewater treatment plants. These substances can cause serious problems, such as making bacteria resistant to antibiotics, causing toxic effects, or disrupting hormones in both the environment and humans. SMX and DLF are commonly found in wastewater and can contaminate soil and plants when this water is reused for irrigation. This research aims to understand how these PPCPs break down and accumulate in soil and plants. We have used earthworms and microbes as indicators to see how these contaminants affect the environment. Additionally, we have studied how two specific plants, *Arabidopsis thaliana* and carrot callus cells, absorb and metabolize these PPCPs. These plants are chosen because they are commonly used in scientific research and are easy to work with. By developing methods to detect these PPCPs and their breakdown products in soil, microbes, and plants, we have found out how these substances move through the environment and how they can be broken down. The ultimate goal is to use these plants to clean up contaminated environments, making them safer for both nature and humans.

Part C: Research Output

7. 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 correspond-ing 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)*						
2022				Pan, Min.*, Hui, L.C., Law, C.M.Y, Auyeung, S.M.	Effects of Composting Yard Waste Temperature on Seed Germination of a Major Tropical Invasive Weed, Leucaena leucocephala / *Sustainability*, 2022. 14, 13638.	No	Yes [Attachment 1]	Yes	Yes
2023				Pan, Min.*, Zhang, H., Luo, L.W. and Yau, P.C.	Exploring the Potential of Co-Application of Sewage Sludge, Chinese Medicinal Herbal Residues and Biochar in Minimizing Human Exposure to Antibiotics Contamination in Edible Crops. / *Sustainability*, 2023. 15, 2980.	No	Yes [Attachment 2]	Yes	Yes
2023				Pan, Min.* Lee, Shing Him Luo, Liwen Chen, Xun Wen Sham, Yik Tung	Co-Application of Sewage Sludge, Chinese Medicinal Herbal Residue and Biochar Attenuated Accumulation and	No	Yes [Attachment 3]	Yes	Yes

					Translocation of Antibiotics in Soils and Crops / *Sustainability* , 2023. 15, 6972.				
2023				He, Yuxin Zhao, Jiusheng Sham, Yik-Tung Gao, Shengjie Pan, Min Chen, Qiaoshan Huang, Guocheng * Wong, Po Keung and Bi, Jinhong	Efficient Hydrogen Peroxide Photosynthesis over CdS/COF for Water Disinfection: The S-Scheme Pathway, Oxygen Adsorption, and Reactor Design / *ACS Sustainable Chem*	No	Yes [Attachment 4]	Yes	Yes
2024				Pan, Min*, Lee, Shing Him, Sham, Yik Tung, Ho Chun Kiu, Zhang Hao	Phytoremediation of diclofenac and sulfamethoxazole in Arabidopsis thaliana cells and seedlings / *Chemosphere*	No	Yes [Attachment 5]	Yes	Yes
2024				Li, Zhenzhen Chen, Xiaoping Huang, Guocheng * Wang, Jianchun Sham, Yik-Tung Pan, Min Bi, Jinhong	Highly porous NiFe-mixed metal oxides derived from calcinated layered double hydroxide for efficient antibiotics removal / *Applied Surface Science*	No	Yes [Attachment 6]	Yes	Yes

8. 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)*
N/A						

9. Whether Research Experience And New Knowledge Has Been Transferred / Has Contributed To Teaching And Learning

(Please elaborate)

I have actively shared my research experiences and the latest knowledge in various teaching courses related to environmental pollutant treatments. These courses include JUPAS Programs such as Environmental Science & Green Management, Life Sciences, and STEM, as well as Postgraduate Programs like Chinese Environmental Studies and Global Environmental Management for Sustainable Development. My primary objective is to deliver high-quality education to our students and equip them with the necessary qualifications, knowledge, and practical skills derived from this study. I have been involved in training undergraduates at HKMU, and it is gratifying to see these individuals evolve into potential research scholars and future professionals in the industry. By imparting the findings and insights gained from this project, I strive to contribute to their development and help nurture their passion for environmental sciences.

10. 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**
	Bachelor of Food Testing Science	2023	04/2023

11. Other Impact

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

We have produced a number of important research outcomes that will provide benefits for HKMU, HKBU, Thei and Fuzhou University, as well as environmental and policy communities concerned with the contamination in Hong Kong. Professionals and practitioners of the agricultural industry can yield useful information on the risk quotients of PPCPs to target plants, through the reuse of treated wastewater and biosolids in agriculture. This information will improve long-term environmental sustainability, soil protection, and impact. Policymakers of the agricultural field can know more about the fate, degradation pathways and bioaccumulation of PPCPs in soil-plant systems, as well as food safety. The information helps them to devise solutions to improve social welfare, social cohesion, and national security.

12. 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	6				Type	No.

13. 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**
N/A	