RGC Ref. No.:
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(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

Effects of sewage sludge-Chinese medicinal herbal residue-biochar amendment on antibiotics and antibiotic resistance genes in agricultural soil and plants

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

Research Team	**Name / Post**	**Unit / Department / Institution**
Principal Investigator	PAN Min / Lecturer	Department of Applied Science, School of Science and Technology, Hong Kong Metropolitan University
Co-Investigator(s)	NA	
Others		

3. Project Duration

	Original	**Revised**	**Date of RGC / Institution Approval** *(must be quoted)*
Project Start Date	01/01/2020	NA	NA
Project Completion Date	30/06/2022	31/12/2022	14/06/2022
Duration *(in month)*	30	36	14/06/2022
Deadline for Submission of Completion Report	30/06/2023	31/12/2023	14/06/2022

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. Study and determine the nutrient level and best proportion of SL-CMHR-BC as a soil amendment;
2. Test the hypothesis that SL-CMHR-BC amendment has an inhibitory effect on antibiotic and ARG bioavailability in soils and crop accumulation;
3. Evaluate the potential impacts of antibiotics contamination on the generation and transfer of antibiotic and ARG bioaccumulation mechanisms in the soil-plant system;
4. Find an effective way to solve antibiotic pollution problems in terrestrial environments and to decrease the ecological risks of ARGs and their potentially adverse effects on human health via the food chain.

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 performance of soils amended with three different proportions (5%, 10%, and 20% on a dry weight basis) of SL-BC and SL-CMHR-BC in terms of improving soil properties and reducing antibiotic levels in the soil-plant system. Carrot and lettuce, two common crop species, were used for the experiment. Overall, the amended soils exhibited higher nutrient levels compared to the control soils, particularly those with 20% SL-CMHR-BC. However, the germination performance of both crop species appeared to be delayed in soils with 10% or 20% SL-BC or SL-CMHR-BC, although this negative effect is likely to diminish over time.

Objective 2: We assessed the impact on antibiotic levels, a total of six target antibiotics (amoxicillin, tetracycline, sulfamethazine, norfloxacin, erythromycin, and chloramphenicol) were applied for crop growth using irrigation water with concentrations of 3 μg/L (IW3) and 30 μg/L (IW30) for each antibiotic. The amended soils demonstrated lower antibiotic levels in both soil and crop tissues compared to the control, with the 20% SL-CMHR-BC soils exhibiting the most significant effect. The study confirms that both SL-BC and SL-CMHR-BC have the potential to serve as effective soil amendments for mitigating environmental dispersal and reducing human exposure risks associated with various antibiotics, particularly the use of 20% SL-CMHR-BC.

Objective 3: The results from real-time quantitative PCR (qPCR) analysis revealed that the abundance of ARGs in soils and lettuce leaves/shoots was significantly reduced by the type of soil amendments (SL-CMHRs-BC > SL-BC > control) and the concentration of antibiotics in the irrigation water. Among the different proportions tested, 20% SL-CMHRs-BC was found to be the most effective in reducing antibiotic concentrations and the corresponding ARGs in this study. Six ARGs (tet A, tet C, tet O, sul I, sul II, and sul III) exhibited relatively higher accumulations in both soil and crop samples, while the remaining five (tet B, tet E, tet M, tet S, tet X) showed lower levels. This study builds upon our previous research, which demonstrated the effectiveness of co-applying SL-CMHRs-BC for mitigating antibiotic contamination in tomato plantations. In this continuation, we sought to evaluate the impact of SL-CMHRs-BC on antibiotic concentrations and their associated ARGs. The antibiotic concentrations in all soil samples showed a strong correlation with the corresponding ARGs ($R^2 > 0.9$). Additionally, the abundance of ARGs in lettuce leaves/shoots was more strongly influenced by the antibiotic concentrations in the leaves/shoots themselves compared to the soils. These findings suggest that the co-application of SL-CMHRs-BC can effectively reduce antibiotic concentrations and the abundance of ARGs in both soils and crops, thereby promoting a safer food system and environment.

Objective 4: We investigated the fate of six antibiotics in a greenhouse soil-plant system using tomato, a fruit crop species. Among the different treatments, the pot containing 20% SL-CMHR-BC exhibited the lowest antibiotic concentrations in both soils and tomato tissues. Norfloxacin was found to be the most abundant antibiotic in the fruits, followed by tetracycline. Furthermore, the pot containing 20% SL-CMHR-BC significantly reduced the bioconcentration factor of the fruit, although the effects on the translocation factor varied. To assess the human health risk associated with consuming carrot, lettuce, and tomato, a combination of current and previous data was utilized. The estimated daily intake indicated a negligible risk to human health in general, as compared to the acceptable daily intake, except for chloramphenicol. By employing a concentration of 20% SL-CMHR-BC, the human exposure risk to antibiotic contamination in edible crops can be minimized.

5.4 Summary of objectives addressed to date

Objectives *(as per 5.1/5.2 above)*	**Addressed** *(please tick)*	**Percentage Achieved** *(please estimate)*
1. Study and determine the nutrient level and best proportion of SL-CMHR-BC as a soil amendment;	√	100%
2. Test the hypothesis that SL-CMHR-BC amendment has an inhibitory effect on antibiotic and ARG bioavailability in soils and crop accumulation;	√	100%
3. Evaluate the potential impacts of antibiotics contamination on the generation and transfer of antibiotic and ARG bioaccumulation mechanisms in the soil-plant system;	√	100%
4. Find an effective way to solve antibiotic pollution problems in terrestrial environments and to decrease the ecological risks of ARGs and their potentially adverse effects on human health via the food chain.	√	100%

6. Research Outcome

6.1 Major findings and research outcome

(Maximum 1 page; please make reference to Part C where necessary)

The study aimed to investigate the performance of soils amended with three different proportions (5%, 10%, and 20% on a dry weight basis) of SL-BC and SL-CMHR-BC in terms of improving soil properties and reducing antibiotic levels in the soil-plant system. Carrot and lettuce, two common crop species, were used for the experiment. Overall, the amended soils exhibited higher nutrient levels compared to the control soils, particularly those with 20% SL-CMHR-BC. However, the germination performance of both crop species appeared to be delayed in soils with 10% or 20% SL-BC or SL-CMHR-BC, although this negative effect is likely to diminish over time. To assess the impact on antibiotic levels, a total of six target antibiotics (amoxicillin, tetracycline, sulfamethazine, norfloxacin, erythromycin, and chloramphenicol) were applied for crop growth using irrigation water with concentrations of 3 μg/L (IW3) and 30 μg/L (IW30) for each antibiotic. The amended soils demonstrated lower antibiotic levels in both soil and crop tissues compared to the control, with the 20% SL-CMHR-BC soils exhibiting the most significant effect. The study confirms that both SL-BC and SL-CMHR-BC have the potential to serve as effective soil amendments for mitigating environmental dispersal and reducing human exposure risks associated with various antibiotics, particularly the use of 20% SL-CMHR-BC.

To investigate the fate and distribution of eleven prevalent antibiotic resistance genes (ARGs) in different environmental settings using various soil amendment treatments at different proportions. Lettuce was cultivated in these soils and irrigated with IW3 or IW30 concentrations of antibiotic-contaminated water to assess the potential spread of antibiotics and corresponding ARGs within the food web. The results from real-time quantitative PCR (qPCR) analysis revealed that the abundance of ARGs in soils and lettuce leaves/shoots was significantly reduced by the type of soil amendments (SL-CMHRs-BC > SL-BC > control) and the concentration of antibiotics in the irrigation water. Among the different proportions tested, 20% SL-CMHRs-BC was found to be the most effective in reducing antibiotic concentrations and the corresponding ARGs in this study. Six ARGs (tet A, tet C, tet O, sul I, sul II, and sul III) exhibited relatively higher accumulations in both soil and crop samples, while the remaining five (tet B, tet E, tet M, tet S, tet X) showed lower levels. This study builds upon our previous research, which demonstrated the effectiveness of co-applying SL-CMHRs-BC for mitigating antibiotic contamination in tomato plantations. In this continuation, we sought to evaluate the impact of SL-CMHRs-BC on antibiotic concentrations and their associated ARGs. The antibiotic concentrations in all soil samples showed a strong correlation with the corresponding ARGs ($R^2 > 0.9$). Additionally, the abundance of ARGs in lettuce leaves/shoots was more strongly influenced by the antibiotic concentrations in the leaves/shoots themselves compared to the soils. These findings suggest that the co-application of SL-CMHRs-BC can effectively reduce antibiotic concentrations and the abundance of ARGs in both soils and crops, thereby promoting a safer food system and environment.

To investigate the fate of six antibiotics in a greenhouse soil-plant system using tomato, a fruit crop species. Among the different treatments, the pot containing 20% SL-CMHR-BC exhibited the lowest antibiotic concentrations in both soils and tomato tissues. Norfloxacin was found to be the most abundant antibiotic in the fruits, followed by tetracycline. Furthermore, the pot containing 20% SL-CMHR-BC significantly reduced the bioconcentration factor of the fruit, although the effects on the translocation factor varied. To assess the human health risk associated with consuming carrot, lettuce, and tomato, a combination of current and previous data was utilized. The estimated daily intake indicated a negligible risk to human health in general, as compared to the acceptable daily intake, except for chloramphenicol. By employing a concentration of 20% SL-CMHR-BC, the human exposure risk to antibiotic contamination in edible crops can be minimized.

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

Further discussions can be initiated with key policymakers, including the Agriculture, Fisheries, and Conservation Department of Hong Kong and the Environmental Protection Department. Engaging policymakers in the agricultural and horticultural sectors would provide them with valuable insights into the application of SL-CMHRs-BC in soil-plant systems, environmental pollutant treatments, and food safety practices. These discussions would foster a deeper understanding of the potential benefits and implications of implementing SL-CMHRs-BC as a sustainable solution in various contexts, enabling policymakers to make informed decisions regarding its adoption and integration into existing policies and practices.

7. Layman's Summary

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

Sewage sludge (SL) is characterized by its high content of organic matter, phosphorus, nitrogen, and microelements. Chinese medicinal herbal residues (CMHRs) consist mainly of fibrous plant materials and are rich in carbon. Additionally, biochar (BC) is known for its positive impact on soil fertility, enhancing the physical, chemical, and biological properties of soils. When SL-CMHR-BC is combined as a soil amendment, it offers the potential to immobilize the bioavailable fraction of certain organic pollutants, heavy metals, and resistant bacteria. This research focuses on determining the optimal nutrient levels and proportions of SL-CMHR-BC as a novel soil amendment. The study aims to prove the hypothesis that the SL-CMHR-BC amendment exerts an inhibitory effect on the bioavailability of antibiotics and antibiotic resistance genes (ARGs) in soils, as well as their accumulation in crops, particularly under conditions of high nutrient levels. Furthermore, the study evaluates the potential impacts of antibiotics on the generation and transfer of antibiotic and ARG bioaccumulation mechanisms within the soil-plant system. The findings of this study provide an effective approach to address antibiotic pollution in terrestrial environments and mitigate the ecological risks associated with ARGs, consequently reducing their potential adverse effects on human health through the food chain.

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)*						
2020				Hoi Yan Chow & Min Pan*	Fertilization Value of Biosolids on Nutrient Accumulation and Environmental Risks to Agricultural Plants, Water Air Soil Pollut (2020) 231:578	Yes (2020)	Yes [Attachment 1]	Yes	Yes
2021				Pan, Min.*, Yau, P.C., Lee, K.C., Zhang, H., Lee,V., Lai, C.Y., Fan, H.J.	Nutrient accumulation and environmental risks of biosolids and different fertilizers on horticultural plants Water, Air, & Soil Pollution, 2021. 232, 480.	No	Yes [Attachment 2]	Yes	Yes
2022				Pan, Min.*, Yau, P.C., Lee, K.C., Man, H.Y.	Effects of different fertilizers on the germination of tomato and cucumber seeds, Water, Air, & Soil Pollution, 2022. 233, 25.	No	Yes [Attachment 3]	Yes	Yes
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	No	Yes [Attachment 4]	Yes	Yes

					Weed, Leucaena leucocephala Sustainability, 2022. 14, 13638.				
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 5]	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 Translocation of Antibiotics in Soils and Crops, Sustainability, 2023. 15, 6972.	No	Yes [Attachment 6]	Yes	Yes
		2024		Pan, Min* Sham, Yik Tung	The effects of sewage sludge-Chinese medicinal herbal residues-biochar on antibiotics and antibiotic resistance genes in soil-plant system	2023	No	Yes	No

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)*
06/2023/ Hong Kong	The effects of sewage sludge-Chinese medicinal herbal residues-biochar to antibiotics and antibiotic resistance genes in soil-plant system	ICSWHK2023: International Conference on Solid Waste 2023: Waste Management in Circular Economy and Climate Resilience (ICSWHK2023)	No	Yes [Attachment 7]	Yes	Yes

10. 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 Thei, 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.

Furthermore, I have taken steps to protect the innovation derived from this project by applying for a patent, ensuring that the intellectual property associated with our research is safeguarded. This will enable us to explore potential commercial applications and further advance the field of environmental pollutant treatments.

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
[redacted]	BA (Hons) Horticulture and Landscape Management, Thei	1/9/2016	31/8/2020 [Attachment 8]

12. Other Impact

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

This study serves as a crucial and foundational cornerstone for the application of SL-CMHRs-BC in soil-plant systems, environmental pollutant treatments, and food safety practices. The findings and insights obtained from this research provide a solid groundwork and essential knowledge that can be utilized to advance the implementation and understanding of SL-CMHRs-BC in various contexts.

By establishing the effectiveness and potential benefits of SL-CMHRs-BC, this study lays the groundwork for further exploration and application of this innovative approach in addressing environmental pollutants, promoting sustainable agriculture, and ensuring food safety. The outcomes of this research contribute to the development of practical solutions and strategies that can positively impact soil quality, pollutant remediation, and the overall sustainability of agricultural systems.

Overall, this study plays a pivotal role in establishing SL-CMHRs-BC as a valuable tool and provides a solid foundation for future research and practical applications in the fields of soil-plant systems, environmental pollutant treatments, and food safety.

The patent application was filed on 11 May 2023.

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	0	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
N/A	