FELLOWSHIP SUMMARY REPORTS

Name of the fellow	Juan C. Sanchez-Hernandez
Home institution	Lab. Ecotoxicology, Fac. Environmental Science and Biochemistry, University of Castilla-La Mancha, 45071, Toledo, Spain
Subject title	Earthworm-assisted bioactivation and ecotoxicity for revalorization of biochars produced from co-pyrolyzing plastic with biomass waste streams
Theme number	Theme III (Transformation Technologies and Innovation)
Contract number	JA00101046
Host institution	Coastal Plain Soil, Water and Plant Conservation Research (United States Department of Agriculture), Florence, South Caroline (USA)
Host collaborator	Dr. Kyoung S. Ro
Dates of the fellowship	15 July to 14 October

I consent to my report being posted on the Co-operative Research Programme's website.

07/12/2019, Toledo (Spain)

1. What were the objectives of the research project? Why is the research project important?

Objectives (O)

O1. **Ecotoxicological assessment** of biochars made from co-pyrolysis of plastic wastes (spent plastic mulch films) with solid organic residues. The aim involved the measurement of toxicity endpoints (mortality, weight change, and biomarkers) in earthworms after incubation in biochar-amended soils.

O2. **Enzymatic bioactivation** of the upgraded biochars (plastic+biomass) using earthworms as biological vectors. This second aim was achieved by measuring enzyme activity in biochar particles recovered from biochar-amended soils incubated with earthworms.

Both aims were initially formulated to respond the following **hypothesis**: "Co-pyrolysis of plastic debris with biopolymer-rich organic wastes generates an upgraded biochar with surface functionalities that facilitate the retention of soil extracellular enzymes". The immediate **prediction** of our hypothesis is that the co-application of upgraded biochar and earthworms increases the persistence and concentration of soil extracellular enzymes for agronomic and remediation purposes.

Interest of the research project

Our research project provided novel findings and potential future applications in sustainable agriculture, waste reduction technologies, and development of bioproducts and bioprocesses, which are some of the key aims in the Theme III of the CPR program. The value-added biochar resulted the best option for increasing soil enzyme activities. Accordingly, we suggest two practical approaches for using this biotechnological approach: "**Vermichar**" (i.e., activation of upgraded biochar during vermicomposting) and "**Lombrichar**" (i.e., co-application of earthworms and upgraded biochar to soil) (Fig. 1). Both strategies may be developed in a circular economy scheme whereby solid organic residues (e.g., cattle manure, pruning remains) and spent mulching plastics are pyrolyzed to yield the value-added biochar. This carbonaceous product may be later ex-situ (Vermichar) or in-situ (Lombrichar) activated depending on the environmental conditions or type of crop. On the other hand, our project also provided promising results in the end-of-life management of **plastic residues**, which is an emerging environmental issue of global concern. Therefore, we think that results of this project (and the practical scheme for its application) respond to sustainable agriculture challenges.

2. Were the objectives of the fellowship achieved?

Or are they on the way to being achieved?

If not, for what reasons? (The data or research is still ongoing or being analysed; technical reasons (e.g. equipment not working, adverse weather conditions, unexpected results, etc.; other reasons?)

Objectives (O)	% achievement	Problems/setbacks	Deliverables (D)
O1 Ecotoxicological assessment	100	Some biochemical biomarkers could not be analysed because technical reasons (long delivery date of reagents and excessive costs). However, analysis of other toxicity endpoints (e.g., weight change, behavior) allowed us to identify suitable upgraded biochars for the objective 2.	D1) Identification of suitable upgraded biochars for in-situ enzymatic bioactivation. D2) Activation of biochar with pesticide-detoxification enzymes. D3) Result dissemination (2 scientific articles[a], 3 conferences[b], 2 seminars[c], 2 master courses[d]).
O2 Enzymatic activation	100	We only used carboxylesterase as a model enzyme for testing biochar activation. The reason was the excessive costs of the other enzymes initially planned (and reagents for enzyme assays).	

		However, the use of purified carboxylesterases obtained from local suppliers allowed us to demonstrate the enzymatic activation of our biochars.	
O3 Biochar activation aging (new objective)	25	This was a new objective derived from unexpected results. Although we performed some experimental trials in the host institution, the aim will be completed (75%) in the Lab of Ecotoxicology (fellow institution) along 2020.	
O4 In situ plastic biodegradation (new objective)	50	This was also a new objective, which was derived from many meetings at the host institute with Dr. Ro and some other scientists. An additional lab experiment using biodegradable plastics will be run in the fellow's lab (Spain) along 2020 to complete the preliminary results obtained in the host institution.	1 scientific article (perspective paper)[e]

Results of our project have been (will be) shared in the following dissemination channels:
[a] #1: Sanchez-Hernandez et al. (2019). Biochar and earthworms working in tandem: Research opportunities for soil bioremediation. Sci. Total Environ. 688:574-583.
#2: Sanchez-Hernandez et al. (2019). Assessing the potential for enzymatic bioactivation of biochars produced by co-pyrolyzing plastic and biomass waste streams. Manuscript written and receiving feedback from the other co-authors at the moment of writing this report (to be submitted to Journal of Hazardous Materials).
[b] One conference in International Visitor Program – Science Leadership Forum, Washington DC, August 12-15, 2019 (The work was presented to audience of US Forest Service and US Department of Agriculture). Two conferences next year: International Congress on Ecological Transitions in Transactions and Actions, 22-26 June 2020, Toulouse (France), (invited speech), and 8th International Conference on Sustainable Solid Waste Management, 17-20 June 2020, Thessaloniki (Greece).
[c] One seminar at the host institute (USDA, exit seminar) to scientists and technical personnel. A second seminar in the Free University of Bolzano (Fac. of Science and Technology, Italy) to scientists and PhD. Students (20/11/2019).
[d] One seminar in the University Master course "Environmental contamination, toxicology and public health" at the University of Valencia, Spain (26/11/2019).
[e] Sanchez-Hernandez JC, Capowiez Y, and Ro K. Earthworm habitats for boosting decay of biodegradable plastics: an ecofriendly end-of-life management option. *ACS Sustainable Chemistry and Engineering* (Ref. sc-2019-054508, R1 version under review).

3. What were the major achievements of the fellowship? (up to three)

Increase of international network	The fact of working in the USDA with Dr. Ro has been a unique opportunity to increase the visibility of my research at the University of Castilla-La Mancha (Toledo).
New research line	Two accidental discovering related to the role of earthworms in the biodegradation of plastics have allow us to start a new research topic related to plastic pollution in soil, in which earthworms could have a notable role in accelerating biodegradation of biodegradable plastics in soil and composting.
Scientific outcome	The visit to USDA, OECD-CRP grant, and the scientific output obtained probably will be of high impact in my academic promotion to full professor.

4. Will there be any follow-up work?

Is a publication envisaged? Will this be in a journal or a publication? When will it appear?	Yes, it is. We have published a scientific paper (*see* table in heading 2), a second paper is under review in its R1 version, and a third paper is getting feedback from co-authors. Our expectation is to have these two papers published by Spring 2020. Moreover, we have been invited to contribute to the special issue entitle "Advances in bioremediation strategies: solving current application limitations and expanding their use to emerging organic contaminants" that will appear in the journal *Applied Science* (deadline for submition: 31 March 2020).
Is your fellowship likely to be the start of collaboration between your home institution and your host?	Yes, absolutely. In fact, USDA is now collaborating in a 3-year research project funded by the Spanish Government (Ref. PGC2018-098851-B-I00). In addition, we have recently won a 2-year research project from the Chilean Government, which has two goals: to apply the biochar technology and its bioactivation in the Chilean agroecosystem, and to initiate a Master course in the University of Concepción (Chile) on "Circular Economy for the Rural Environment".
Is your research likely to result in protected intellectual property, novel products or processes?	Probably yes, although we have still to continue the work during next year to ensure the preliminary results at the host institution.

5. How might the results of your research project be important for helping develop regional, national or international agro-food, fisheries or forestry policies and, or practices, or be beneficial for society?

Please express this in terms of environmental/food security/food safety/economic/health (human and livestock and plant) benefits, etc.

Environmental and food security benefits	It is widely accepted that biochar is a viable option of fighting against climate change because of carbon sequestration in the own biochar structure, and reduction of greenhouse gases emissions from soil when applied as an amendment. In our project, we have demonstrated that the pyrolysis of blended plastic residues and solid organic wastes yield a value-added biochar with a stronger capacity to bind soil enzymes than biochar produced from biomass alone. Therefore, this strategy opens a balcony of technological possibilities in which earthworms (and microorganisms) may be used to increase the functionality of biochar for improving soil fertility or removing (inactivate) pesticides (Fig. 1). Moreover, the possibility of obtaining an upgraded biochar from plastic debris is undoubtedly an option to alleviate the input of non-biodegradable, polyethylene-based plastic debris into the environment.
Economic benefits	The use of upgraded biochars as suggested in Fig.1 could mean a significant reduction of irrigation water, because of the beneficial impact of biochar on soil hydrodynamic. Furthermore, the increase of soil enzymes in biochar-treated soils would lead to stimulating nutrient cycles, and probably to increase the available fraction of nutrient to plants, thus reducing the use of fertilizers.

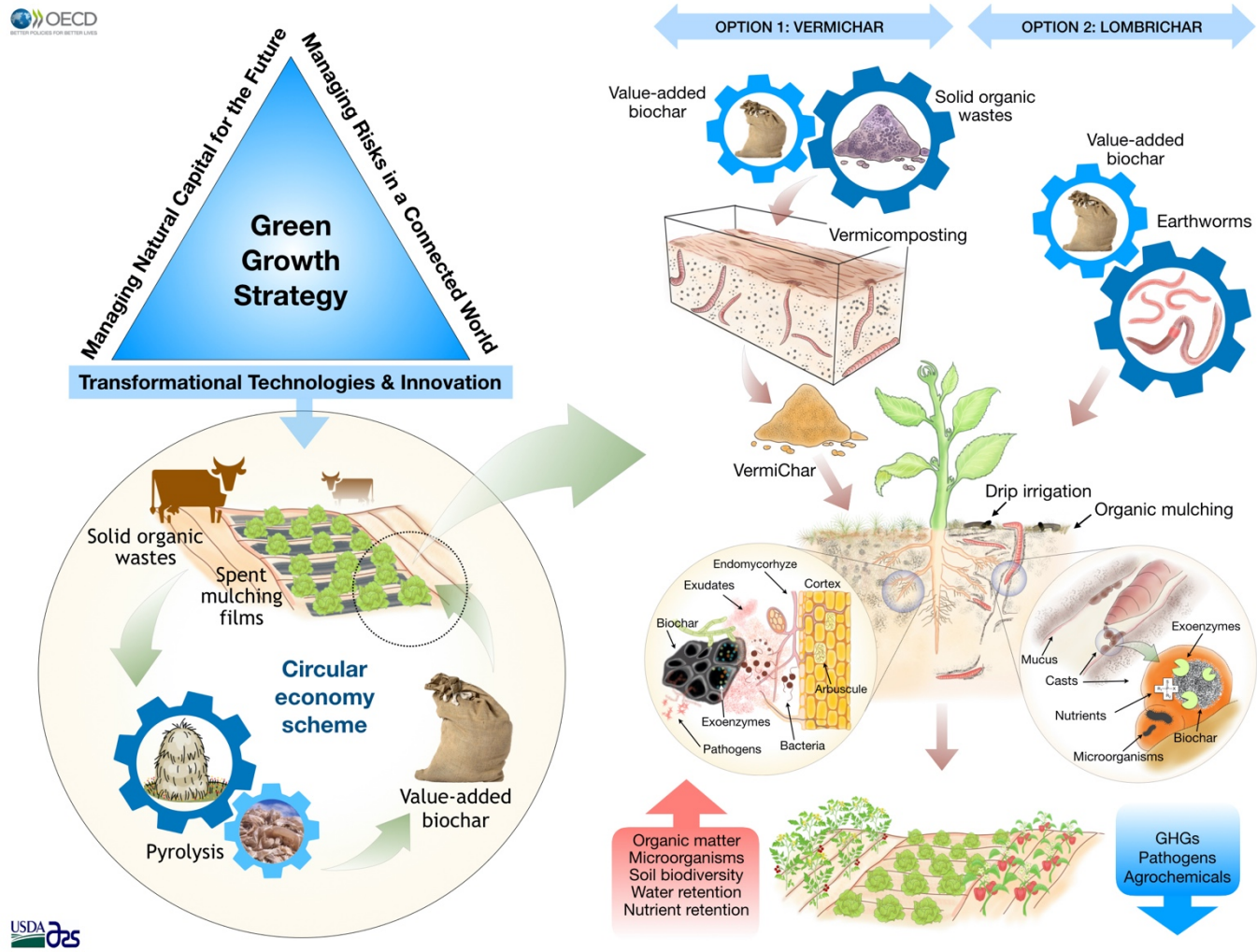

Figure 1. Conceptual model of circular economy with biochar. Value-added biochars produced from blended plastic debris and solid organic wastes are introduced in agriculture as a potential pro-fertilizer, which must be enzymatically activated by treatment with earthworms. This biotechnological system suggests two practical approaches: vermicomposting of the value-added biochar mixed with solid organic waste (Vermichar approach), and co-application of value-added biochar and soil-dwelling earthworms (Lombrichar approach). The biochar-linked circular economy scheme is connected with the Theme-III of the CRP operative scheme (taken from the CRP Research Themes 2016-2020, page 1). This model of sustainable agriculture based in the use of earthworms and biochar has been recently published (Sanchez-Hernandez et al. 2019, *Science of the Total Environment*, 688:574-583).

6. Satisfaction

Did your fellowship conform to your expectations?	Yes, absolutely. My research activity and funding opportunities have increased significantly in the field of biochar technology.
Will the OECD Co-operative Research Programme fellowship increase directly or indirectly your career opportunities? Please specify	Yes. Promotion to full professor (pending).
Did you encounter any practical problems?	No.
Please suggest any improvements in the Fellowship Programme.	I found the CRP program optimum.

7. Advertising the Co-operative Research Programme

How did you learn about the Co-operative Research Programme?	Seeking grants for senior researchers on Internet.
What would you suggest to make it more "visible"?	Perhaps, diffusion of the annual calls in some specific scientific journals related to the scope of CRP.
Are there any issues you would like to record?	No.