Microbiological strategies for agronomic biofortification of crops for micronutrient acquisition.

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Project background: Many human and animal micro-nutritional requirements are obtained through consumption of shoots, grains and seeds via accumulation from the soil (Ali et al., 2010). Soils deficient in such trace metals or where the metal exists in non-bioavailable forms lead to nutritional deficiencies, prompting calls for so-called agronomic bio-fortification where the required micronutrient is added to the soil along with major nutrient fertilisers (e.g. (Broadley et al., 2006, Chilimba et al., 2012). Such approaches have recently been suggested for improving zinc and selenium deficiency and related diseases in Malawi (Hurst et al., 2013). However, fertiliser supply is limited (mineral phosphorus reserves are predicted to run out by the end of the 21st century) and fertilisers are not always affordable, especially in low income countries and also come with the known problems of freshwater eutrophication.

Meanwhile, microbial inoculation of seeds and/or soil is a well-known mechanism for improving nutrient availability and acquisition from soil with detectable improvements in crop yield. However whether this also improves the bioavailable fraction of nutrients in edible parts is unclear (Sebastian et al., 1998), especially as some studies have shown micronutrient enrichment in husk which means they get lost during processing (Singh et al., 2013). Significantly, studies have shown that some bacteria promote plant growth and resistance to toxic metals when seed or soil is inoculated with these so-called plant growth promoting bacteria (PGPBs). This growth promotion has been linked to a variety of mechanisms, including prevention of excessive secretion of ethylene in plants, optimum production of plant essential hormones (e.g. cytokinins and gibberellins), improved fixation, release and utilization of essential nutrients, and change in metal speciation (Khan et al., 2009).

Using glass house experiments with oil seed rape (Brassica juncea), we have recently shown (Adediran et al. 2014) that inoculation of seeds/soil with the rhizospheric Rhizobium leguminosarum and endophytic Pseudomonas brassicacearum PGBP led to improved growth of the plant under zinc contamination while also removing more of the zinc from the soil. Paradoxically, spectroscopic studies showed that inoculated plants accumulated more zinc in their roots (Figure 1a), a result that was linked to zinc sequestration in the form of phytate (Figure 1b). However, there was no opportunity to analyse above ground tissues for Zn distribution, which raises several questions: (i) Is zinc translocated to aerial parts and, if so, does speciation change between root and shoot tissues? (ii) Does the same mechanism operate for other
micronutrient metals? (iii) Can the mechanism be exploited to improve micronutrient uptake and translocation to edible parts? (iv) Are the micro-nutrients in bioavailable form in edible parts and hence can this approach be a viable alternative to chemical agronomic biofortification? This project will address these questions by characterising the distribution, concentration and speciation of one micronutrient metal (Zn) and one micronutrient metalloid (Se).

Methodology: We will perform plant growth experiments focussing on tracking exchanges and differences between roots, shoots and seeds of edible crops with and without inoculation with PGPB. Two plants (oil seed rape and rice or maize) will be compared. We note that complexation with phytate is known to render some metals unavailable (Lönnerdal et al., 2011), hence being able to establish changes in speciation between roots and shoots/seeds is critical. Focus will be on establishing whether bacteria induce bioaccumulation of Zn and Se in rice/maize roots and seeds from soils with different bioavailability characteristics (low metal concentrations, strongly fixed forms, pH characteristics, redox status which is key for different growth phases for rice). These studies will be complemented by growth of rice/maize in contaminated media (spectroscopic analysis requires high tissue concentrations) combined with spectroscopic analysis to determine speciation. The research timetable is outline in the gantt chart below.

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Training: A comprehensive training programme will be provided comprising both specialist scientific training and generic transferable and professional skills. Specific skills acquired during this project will include synchrotron based X-Ray absorption spectroscopy at Diamond Light Source Ltd with associated data analysis and modelling packages to derive speciation and structural information. The student will attend a general course on synchrotron radiation as well as a specific course on spectroscopic data processing. The student will also be trained in microscopic and imaging techniques microbial culture and isolation and well as plant growth methods. Training will be given in statistical packages for analysing growth data for which training will be given.

Requirements: Applicants should have a natural science background and especially biological, agricultural, environmental chemistry and environmental/earth sciences, with a 2:1 degree and above.

References
CHILIMA, B. & GONDWE, J. 2013. Soil-type influences human selenium status and underlies widespread selenium
deficiency risks in Malawi. Scientific reports, 3.
Genotypes of Maize (Zea mays L.), Barley (Hordeum vulgare L.), and Rice (Oryza sativa L.) Assessed in a Suckling
SEBASTIAN, S., TOUCHBURN, S. P. & CHAVEZ, E. R. 1998. Implications of phytic acid and supplemental microbial phytase in

**Project summary:** This research will explore whether inoculation of food crops with plant
growth promoting bacteria is a sustainable method for bio-fortification of food stuffs to
improve diets worldwide.