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Change in Root Metal Speciation and Compartmentalization are Complementary Plant Growth Promoting Mechanisms by Bacteria

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Short Communication

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ABSTRACT

The scientific knowledge of below ground root-bacteria-metal system is still significantly limited. A unique study based on the use of synchrotron X-ray absorption spectroscopy (S-XAS) to determine the accumulation and speciation of zinc (Zn) in roots of *Brassica juncea* exposed to Zn contamination following inoculation with *Pseudomonas brassicacearum* and *Rhizobium leguminosarum* provides robust evidence that bacteria ameliorate Zn toxicity by sequestering Zn in compounds that reduce its bio-toxicity and at sites devoid of Zn sensitive organelles.

The inoculation of metal-remediating plants with plant growth promoting bacteria (PGB) has been observed to promote growth while increasing metal accumulation in plants under metal contamination; a process that could be further explored in developing ‘green’ remediation technology for metal contaminated environments [1,2]. However, the mechanisms behind enhanced plant growth in the face of high toxic metal bioaccumulation remain poorly-known.

While research to identify the role(s) that PGB perform in plant-microbe symbiotic relationships in contaminated environments is ongoing worldwide, symbiotic nitrogen fixation, essential plant macro and micro nutrient release, protection of plants against pests and diseases, microbial metal sorption and maintenance of optimum level of phytohormones secretion has been suggested has possible means through which PGB promotes growth and metal accumulation in plant under metal toxicity [3,4].

However, studies conducted in metal contaminated media where the essential plant nutrients and growth hormones are provided and under experimental conditions free of plant pests and diseases have reported reductions in metal toxicity in plants inoculated with PGB. These observations have been linked to possible changes in metal speciation induced by bacteria and this is being regarded as a major plant growth promoting mechanism [5-7]. Although these studies provided valuable insights towards the understanding of the roles of PGB in metal sequestration, they mostly utilized destructive analytical techniques (like chemical extraction of hormones, enzymes and nutrients from bacteria and plants) to study bacteria, metal, and plant as three different units and the likelihood of metal speciation changing during these destructive sample preparation method are very high [3,6,8-11]. The bacteria-metal-plant system is however a dynamic system that needs to be studied as a unit under minimal disturbance in order to properly identifies the roles of PGB in metal translocation, sequestration and possible changes in speciation in plants.

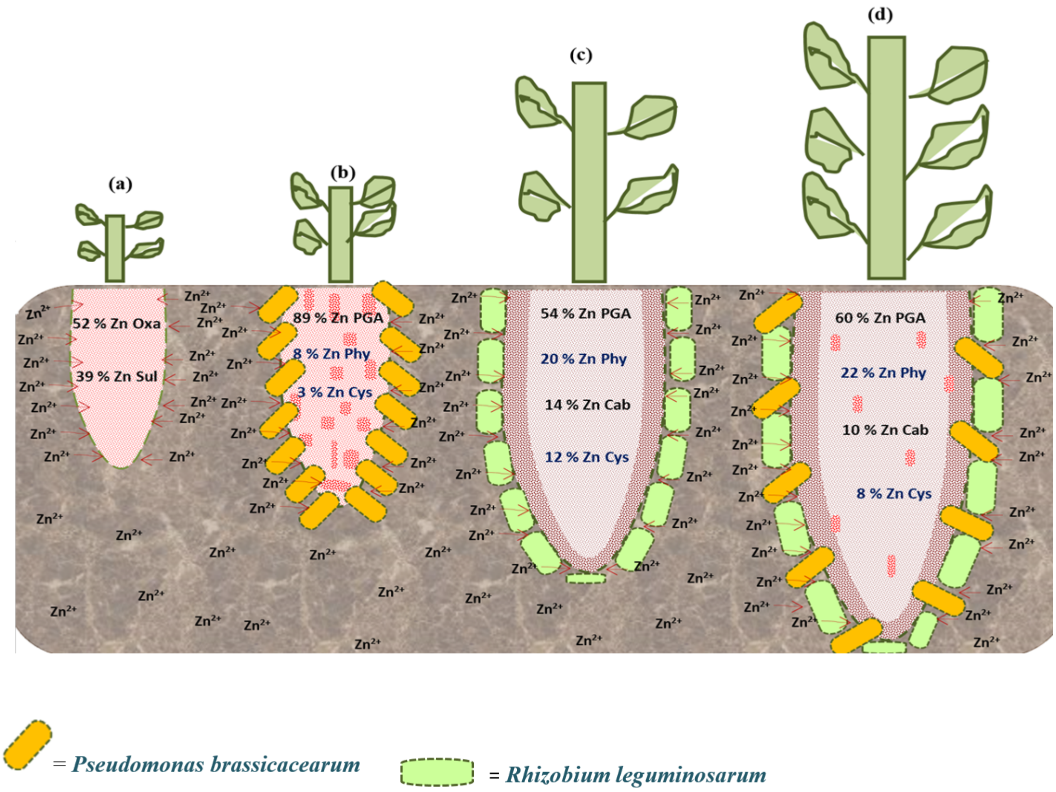
To investigate possible changes in metal speciation/coordination within the plant-microbe-metal system, a factorial, fully replicated plant growth experiment was conducted in a glasshouse at the University of Edinburgh UK by planting *Brassica juncea* (a well-known metal accumulating plant) inoculated with *Pseudomonas brassicacearum* (an endophyte isolated from a Brassica plant) and *Rhizobium leguminosarum* (a rhizophyte isolated from a clover plant), on soil contaminated with 400 mg kg^{-1} Zn (the most widely studied metal contaminant) added as Zn sulphate [12].

To ensure minimum disruption in bacteria-metal-plant system, live plants were transported to the Diamond Light source; UK's national synchrotron science facility, at 5 weeks after planting. Root strands were immediately subjected to combination of Synchrotron based micro-X-ray Fluorescent Imaging (μ-XRF) to study nature of Zn accumulation and micro-X-ray absorption near edge structure (μ-XANES) spectroscopy to probe Zn speciation in the plant roots [1].

Although bacteria inoculations significantly enhanced Zn accumulation, *P. brassicacearum* exhibited the poorest plant growth promoting ability, while *R. leguminosarum* alone and in combination with *P. brassicacearum* significantly enhanced *B. juncea* growth and Zn bioaccumulation [1].

A subcellular analysis of plant root by µ-XRF imaging showed that the PGB enhanced tolerance to Zn contamination by enhancing epidermal Zn compartmentalization depending on the nature of root colonization [1,12]. In plants without root nodules (like *B. juncea*), *R. leguminosarum* mainly resides at the rhizosphere of the plant roots [12,13]. *P. brassicacearum* on the other hand, was isolated from the root of a Brassica plant and has been shown to be capable of colonizing interior root areas [12,14]. *R. leguminosarum* therefore appear to mediate contact of Zn with Zn sensitive organelles in the plant roots by ensuring only enhanced accumulation of Zn at the rhizosphere of *B. juncea* root [1,12].

Furthermore, µ-XANES analysis showed that reduced plant growth in un-inoculated plants and plant inoculated with *P. brassicacearum* was due to root accumulation of Zn as Zn sulphate, Zn oxalate and Zn polygalacturonic acids; forms that has been reported to be toxic to plant growth. Whereas, the better growth and increased metal accumulation observed in plants inoculated with *R. leguminosarum* and its combination with *P. brassicacearum* was attributed to root storage of Zn in the chelated and less toxic forms of Zn phytate and Zn cysteine [1,12]. A conceptual model illustrating this newly observed phenomenon is presented in **Figure 1.**



Zinc forms in root:
Zn Oxa - Zn oxalate, Zn sul – Zn sulphate, Zn PGA – Zn polygalacturonic acid, Zn Cab –Zn carbonate,
Zn Phy – Zn phytate, Zn His - Zn histidine, Zn Cys – Zn cysteine

(a) *Brassica juncea* plant root not inoculated with bacteria: Stunted plant growth and low Zn bioaccumulation attributed to root storage of Zn as Zn oxalate and sulphate

(b) *B. juncea* plant root inoculated with endophytic *P. brassicacearum*: Inoculation with the PGB enhanced Zn bioaccumulation. The dispersed Zn hot spots in the root and storage of Zn mainly as Zn polygalacturonic acid may be responsible for reduced plant growth

(c) *B. juncea* plant root inoculated with rhizospheric *R. leguminosarum*: Inoculation with the PGB significantly enhanced Zn bioaccumulation more than (a&b). The sub-cellular compartmentalisation of Zn at the root epidermis where the PGPB colonised the root and storage of Zn as Zn phytate and Zn cysteine are likely responsible for the high Zn bioaccumulation and better plant growth

(d) *B. juncea* inoculated with *P. brassicacearum* and *R. leguminosarum*: Dual bacterial inoculation conferred multiple growth promoting effects under Zn contamination. Plant growth and Zn bioaccumulation was significantly higher than in all other treatments

Figure 1. A conceptual model illustrating the influence of *P. brassicacearum*, *R. brassicacearum* and a combination of the two on Zn speciation and compartmentalization in the root of *B. juncea* under Zn sulphate.

Inoculation of metal accumulating plants with bacteria that are genetically modified to improve their ability to secrete phytochelatins, and the use of transgenic plants are active areas of phytoremediation research [15,16]. Apart from providing new insights to the mechanisms of enhanced growth under metal toxicity, a microbial-phytoremediation system that combines the use of two or more bacteria on a metal remediating plant, is being suggested as a more affordable and sustainable alternative for remediation of soils contaminated with toxic metals. Nevertheless more studies are required with different bacteria combinations and other metal contaminants to confirm this assertion.

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