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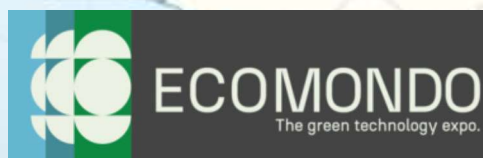
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Non-flavonoid root exudates shaped the plant ‘cry-for-help’ under polychlorinated biphenyl stress

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ABSTRACT

Background information. Soil pollution represents one of the main causes of biodiversity and fertility loss. Polychlorinated biphenyls (PCBs) are xenobiotics with carcinogenic and recalcitrant properties that represent a harm for ecosystem health. Their removal can be achieved through rhizoremediation, a process relying on the beneficial interaction between plants and degrading microorganisms, acting as an holobiont. The holobiont dynamics in polluted soils are poorly understood and may depend on the plant “cry-for-help”, a pollutant-driven shift in the root exudation profile that sustains the microbial services for the contaminant clean-up. Flavonoids are acknowledged as key factors in influencing root chemistry under polychlorinated biphenyls (PCBs) stress, acting as inducers of the bacterial *bph* catabolic operon.

Methods. To decipher flavonoids role in supporting the holobiont fitness under PCB stress, a multidisciplinary approach was adopted, including high-throughput 16SrRNA amplicon sequencing, metabolomics and plant-microbe interaction assay.

Main results. 16S rRNA amplicon sequencing results indicated that *Arabidopsis* plants with an altered pattern of flavonoid exudation (WT, *tt4* null producer, *tt8* and *ttg* over-expressing lines) grown in a PCB-historically contaminated soil differentially affected the structure of the rhizosphere associated microbiome, while the abundance of the *bph* gene estimated by RT-qPCR was similar among the different *Arabidopsis* lines. Further evidence showed that flavonoids supplied as pure chemicals could promote *in vitro* the recruitment and persistence in the root system of the PCB degrader *Paraburkholderia xenovorans* LB400, by improving its proliferation, swimming motility, chemotaxis and biofilm formation. Indeed, early root colonization was enhanced in the flavonoid over-accumulating *Arabidopsis* mutant line *tt8*. Moreover, 100 µM flavone and quercetin activated the transcription of the PCB catabolic *bphA* gene, further supporting the degrading functionality of strain LB400. The bacterium efficiently colonized *Arabidopsis* roots and showed plant growth promoting effects under control conditions and in presence of 20µM PCB-18. Nevertheless, these beneficial effects were not affected in *Arabidopsis* mutant lines with altered flavonoid exudation profiles, leading to the conclusion that non-flavonoid REs might be involved in later stages of plant-microbe interactions under PCB stress. Given these findings, an untargeted metabolomic approach was performed to identify the PCB-driven signature in root chemistry. Among the identified metabolites, flavonoids were not retrieved. Notably, the relative concentration of scopoletin decreased in the REs of PCB-stressed plants: our study reveals that this effect may be due to the antimicrobial ability of this compound that inhibited the growth of the PCB degrader strains *Acinetobacter* P320 and *Pseudomonas* JAB1. The over-exuded metabolites consisted in the primary metabolites hypoxanthine, L-arginyl-L-valine, and L-seryl-L-phenylalanine, and were preferentially used as nutrients and growth-stimulating factors, showing a variable ability to affect rhizocompetence traits like motility and biofilm formation.

Conclusions. These results suggest that the plant ‘cry-for-help’ under PCB stress may reshape the root chemistry to provide more suitable growth conditions for degrading strains and that flavonoids may be less crucial metabolites than thought.

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