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## Side effects of pesticides in groundwater: impact on bacterial denitrification

Caroline MICHEL<sup>1</sup>, Nicole BARAN<sup>1</sup>, Laurent ANDRE<sup>1,2</sup>, Catherine JOULIAN<sup>1</sup>

<sup>1</sup> BRGM, DEPA (Direction de l'Eau, de l'Environnement, des Procédés et Analyses), 3 avenue Claude Guillemin, BP 36000, 45060 Orléans cedex 2 (France)

<sup>2</sup> Université d'Orléans, CNRS, BRGM, UMR 7327 Institut des Sciences de la Terre d'Orléans, 45071 Orléans, France

The increasing pesticide use in agriculture involves the presence of both parent pesticides and their transformation products (metabolites) in various environmental matrices and notably groundwater. One drawback is that pesticides and metabolites (even referred to as emerging groundwater contaminants) are now known to have side effects on non-target surface and subsurface living organisms. The environmental consequence of side effects of pesticides and their metabolites on microbial ecosystems is that they can threaten the ecosystem services based on microbial activities in soil (litter degradation, plant growth, nutrient cycling, degradation of pollutants...) and groundwater (production of drinking water, nutrient cycling, degradation of contaminant...).

Most studies on side effects of pesticides and their metabolites have been conducted in soils, showing main impacts on microbial abundance, presence or absence of microbial species, increase or decrease in gene expression (mainly linked to the N cycle), and increase or decrease of the functional diversity (activities linked to P, N, S, C cycles). Moreover, pesticides presence was shown to usually lead to the selection of microorganisms having the ability to degrade them. Pesticides and their metabolites impacts on soil microbiology are rather well documented, but knowledge on their side effects on groundwater microbial ecosystems is scarce. The few studies conducted in groundwater have underlined potential side effects on groundwater communities as pesticides presence could increase microbial biodiversity. The role of pesticides contamination history on pesticides impact on groundwater communities has also been underlined.

In this context, the impact of two pesticides, S-metolachlor and Propiconazole, and their metabolites, ESA-metolachlor and 1,2,4-triazole, was studied on microbial denitrification, a key function for nitrate removal in groundwater. Laboratory experiments were performed with or without pesticides or metabolites at 2 and 10 µg/L. Kinetics of nitrate reduction along with nitrite and N<sub>2</sub>O production all suggest that S-metolachlor has no or only little impact, whereas its metabolite ESA-metolachlor inhibits denitrification by 65 % at 10 µg/L. Propiconazole and 1,2,4-triazole also inhibit denitrification (by 29-38 %) at both concentrations, but to a lesser extent than ESA-metolachlor. When inhibition occurs, substances affect the reduction of nitrate into nitrite. At the end of batch experiments, no significant change in *narG* and *napA* genes abundance was detected, suggesting an impact of pesticides at the protein level rather than on bacteria abundance. Community diversity fingerprints and Illumina sequencing indicate no major impact of pesticides on bacterial diversity except for ESA-metolachlor at 10 µg/L that induces an increase of biodiversity indices. General growth parameters such as bacterial biomass and acetate consumption suggest no impact of pesticides, except for propiconazole at 10 µg/L that partially inhibits microbial metabolism (as acetate uptake). In conclusion, pesticides and their metabolites can have side effects on microbial denitrification in groundwater at realistic environmental concentrations, and may thus affect ecosystem services based on microbial activities.

**Keywords:** denitrification; groundwater; microbial inhibition; pesticides; metabolites; side effects; laboratory experiments.