Acclimatisation strategies in gastropods to environmental pollutants: response of *L. stagnalis* to toxic cyanobacteria and pesticide exposure

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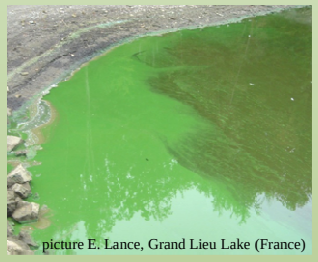
Long-term field investigations in western France evidenced declining populations of indigenous gastropod species, e.g., *Lymnea stagnalis*, whereas the invasive *Physa acuta* became the single occurring gastropod species. The environmental stress enforcing these alarming biodiversity decreases is hypothesised to be a combination of anthropogenic pollution (e.g., pesticides) together with recurrent cyanobacterial blooms producing various toxins [e.g., neurotoxins, hepatotoxic microcystins (MCs)]. MCs and pesticides cause toxic stress to organisms. This study reports the MC accumulation and the physiological reactions of biotransformation and oxidative defence in *L. stagnalis* under exposure to a mixture of non MC-producing and MC-producing cyanobacteria *Planktothrix argardhii* with or without glyphosate in Round Up®.



picture E. Lance, Grand Lieu Lake (France)

Material and methods

Experimental design

• During 3 weeks (intoxication period), *L. stagnalis* fed *ad libitum* with lettuce was exposed in:

1) dechlorinated water **"C"**, **2)** dechlorinated water with Round Up® (1 µg L^{-1}) **"C+R"**, **3)** non MC-producing *P. agardhii* suspension **"MCfree"**, **4)** non MC-producing *P. agardhii* suspension with Round Up® (1 µg L^{-1}) **"MCfree+R"**, **5)** MC-producing (33 µg MCs L^{-1}) *P. agardhii* suspension **"MCprod"**, **6)** MC-producing (33 µg MCs L^{-1}) *P. agardhii* suspension with Round Up® (1 µg L^{-1}) **"MCprod+R"**,

• After this period, snails were placed into dechlorinated water and fed *ad libitum* with lettuce during 2 weeks (depuration period).

Analyses

• Activities of biotransformation (Glutathione-S transferase: GST) and antioxidant (Catalase) enzymes have been followed from 5 digestive glands and 5 feet of gastropods sampled after 1, 3, 7, 14 and 21 days of exposure and after 3, 7, and 14 days of depuration.

• MCs were analysed via Lemieux oxidation of snail tissues, that released the MMPB (2-methyl-3-methoxy-4-phenylbutiric acid) part of the MCs (free and bound to proteins), detected by LC-MS/MS.

• Statistical analyses were performed with R version 2.14.

Results and Discussion

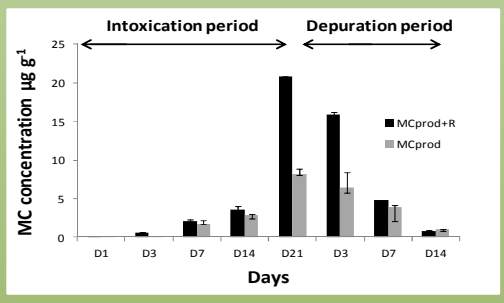


Fig. 1: Total (free and bound) MC accumulation (µg g^{-1}) in digestive glands of gastropods

MC accumulation increased linearly in treatments exposed to MC-producing cyanobacteria during the intoxication, decreasing during the depuration. The addition of pesticide induced an higher MC accumulation at the end of intoxication.

Lymnaea stagnalis

Cytosolic fractions of GST and catalase activities were significantly higher than microsomale fractions in the foot and digestive gland of snails. Activities of both enzymes were significantly higher in the digestive glands vs foot of snails (Fig. 2)

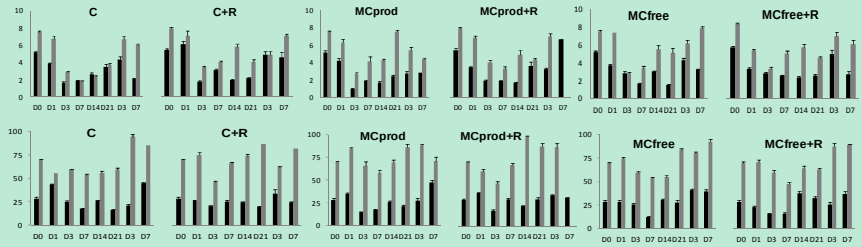


Fig. 2 : Enzymatic response of cytosolic A) glutathione-S transferase [mkat mg^{-1} protein] and B) catalase [mkat mg^{-1} protein] in the digestive gland (grey) vs foot (dark) tissues of *L. stagnalis* exposed to various treatments (see M&M)

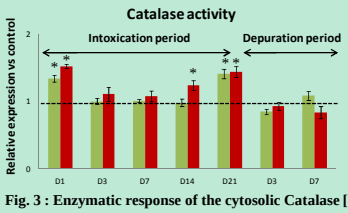


Fig. 3 : Enzymatic response of the cytosolic Catalase [mkat mg-1 protein] and GST [mkat mg-1 protein] in the digestive gland of L. stagnalis exposed to non MC-producing (green) and to MC-producing (red) cyanobacteria. Significant increase compared to the control group (*).

No difference occurred between snails exposed to MC-producing and free MC cyanobacteria. In both treatments: - early induction of catalase occurred after 24h and later one after 21 days - increased GST activity from day 7 of intoxication, appeared exhausted at day 21 and decreased to control values at depuration

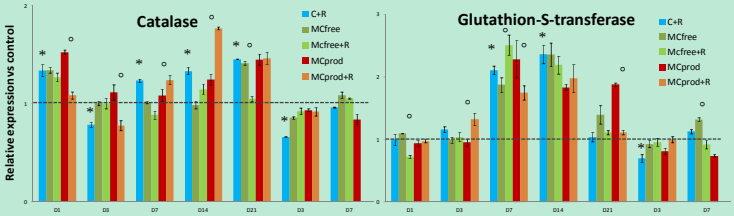


Fig. 4 : Enzymatic response of the cytosolic Catalase and GST in the digestive gland of *L. stagnalis* exposed to non MC-producing and to MC-producing cyanobacteria with or without pesticide Round Up®. Significant increase compared to the control group in Round Up® treatments (*), significant differences between cyanobacterial treatments with and without Round Up® (°).

Compared to control Round Up® induced similar significant effects than cyanobateria (see Fig. 2). The addition of Round Up® to cyanobacteria significantly modified the enzymatic response compared to cyanobacteria alone but with inversed tendencies depending on time.

Conclusions

→ MC kinetics in snail digestive gland showed a linear MC accumulation, concomitant to a high activity of the biotransformation enzyme GST.

→ Anti-oxydant response is induced faster than biotransformation one, the second induction after 21 days of intoxication probably corresponded to exhaustion of GST and resulting oxidative stress in digestive cells.

→ The Round Up alone, at concentration considered as safe for environment (1 µg L^{-1}) , induced an oxidative stress in gastropods, but no clear synergistic effect have been demonstrated when administered with cyanobacteria.

→ Other bioactive compounds than MCs produced by cyanobacteria (e.g., BMAA) may induce anti-oxidant and biotransformation responses in gastropods.

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