Combined effects of heavy metals and soil properties on mesotrione biodegradation by *Bacillus megaterium* Mes11

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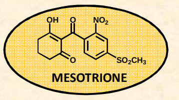
Aim of our study

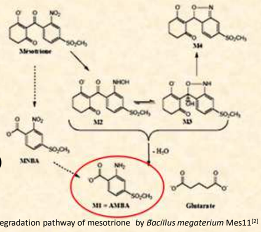
The **increasing soil contamination** with **heavy metals** - coming from the long-term use of phosphate fertilizers, the spread of sludge from wastewater treatment plants etc - and **pesticides** reduces agricultural soil quality and poses important environmental and toxicological problems. Indeed, these heavy metals can accumulate in soil fractions and affect not only the physico-chemical transfer processes but also the soil biological activities. The **aim of our program** is to better understand the effect of heavy metals (Ni^{2+}, Cu^{2+}, Zn^{2+}, Cr^{3+}, Pb^{2+}) on pesticide fate and microbial degrading-activity in soils. A first step focus on the herbicide mesotrione biodegradation by a pure bacterial strain, *Bacillus megaterium* Mes11, isolated from an agricultural soil[1].

Strategy and Materials

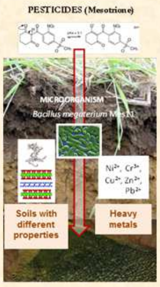
The herbicide Mesotrione



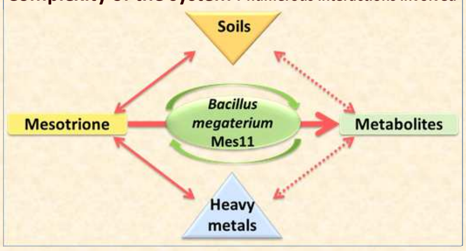
- Selective herbicide on maize
- Belongs to the triketone family
- Commercialized by Syngenta (2003)
- Weak acid pKa = 3.12 (20°C)
- Toxic for aquatic organisms



Biodegradation pathway of mesotrione by *Bacillus megaterium* Mes11[2]



Complexity of the system : numerous interactions involved



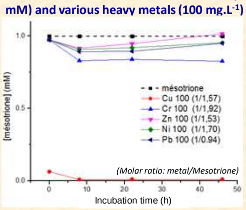
Characterization of soils

Soil	pH H_2O	$CaCO_3$ %	CEC, cmol/kg	Sand, %	Silt, %	Clay, %	OC %	Fe_2O_3 %
Chernozem	7.78	2.72	73.4	7.48	59.9	32.6	2.91	5.13
Luvisol	6.70	0.66	14.6	27.8	54.2	18.0	1.50	3.12
Red soil	5.83	1.93	50.9	28.7	47.0	24.3	0.56	9.31
Vertisol	7.94	11.10	49.8	29.0	51.0	20.0	1.71	4.94

Metal-mesotrione Interactions

Incubation of mesotrione and heavy metals

Kinetics of reaction between mesotrione (1 mM) and various heavy metals (100 mg.L^{-1})



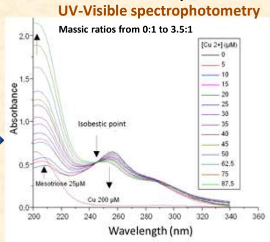
3 different behaviours observed according to the complex stability:

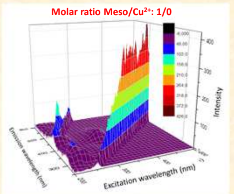
- **Zn^{2+}, Ni^{2+}, Pb^{2+}:** Reversible dissipation of mesotrione (10.5%) within the first 10h and complete release after 45h.
- **Cr^{3+}:** Irreversible dissipation of mesotrione (17%) within the first 10h.
- **Cu^{2+}:** Formation of a stable mesotrione/Cu^{2+} complex and precipitation as Cu hydroxide.

Spectra of Mesotrione-Cu^{2+} complex

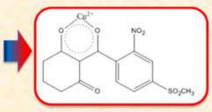
UV-Visible spectrophotometry

Massic ratios from 0:1 to 3.5:1

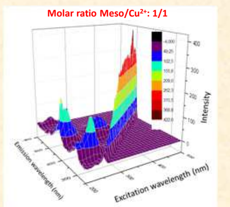




Formation of a 1:1 Mesotrione- Cu^{2+} complex

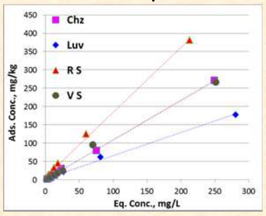


3D Fluorescence spectroscopy



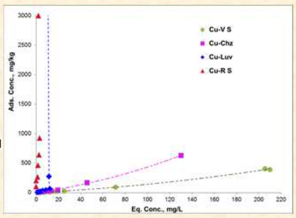
Adsorption of mesotrione on bulk, clay and Cu-contaminated soils

Adsorption isotherms of mesotrione by bulk and Cu-contaminated soils



Experimental conditions

Ratio solid/liquid: 1 /3
Cu-contaminated soils: 2 g.kg^{-1}
Soil mass: 1g
Mesotrione concentration: 0-1000 µM
Stirring at 21° C and 60 rpm for 24h



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Bulk Soil (Clay)	K_F mg kg^{-1}/mg L^{-1}	n_f	r^2	K_d, L kg^{-1} (mean)	K_{OC} (mean)	Adsorbed, % (mean)
Chernozem	1.025 (1.417)	0.981 (0.959)	0.992 (0.999)	1.09 (1.60)	37 (47)	27 (35)
Luvisol	1.356 (1.605)	0.998 (0.983)	0.983 (0.996)	0.74 (1.69)	50 (44)	20 (36)
Red Soil	3.649 (3.604)	1.167 (1.126)	0.996 (0.996)	2.58 (2.79)	464 (276)	46 (48)
Vertisol	1.133 (1.125)	0.949 (0.965)	0.996 (0.996)	1.04 (1.26)	61 (59)	26 (30)

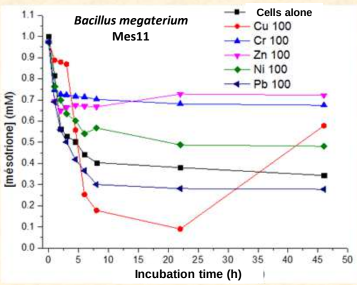
Not linear

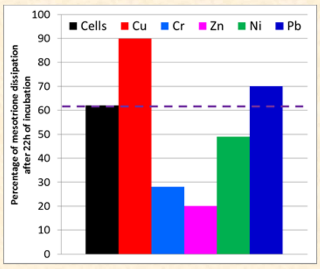
Contaminated Soils	K_F mg kg^{-1}/mg L^{-1}	n_f	r^2	K_d, L kg^{-1} (mean)	K_{OC} (mean)	Adsorbed, % (mean)
Cu-Chernozem	-	-	-	3.32	114	50
Cu-Luvisol	-	-	-	27.98	> 1000	74
Cu-Red Soil	-	-	-	> 1000	> 1000	94
Cu-Vertisol	2.068	0.795	0.990	1.18	69	27

- Variable affinity of the soils for mesotrione – Greater affinity of clay fraction
- Increased adsorption capacity of the Cu-contaminated soils toward mesotrione (except for Vertisol)

Impact of heavy metals on mesotrione biodegradation

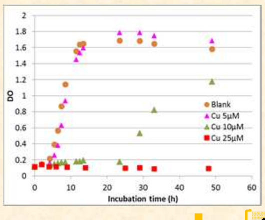
- Kinetics of mesotrione biodegradation in the presence of heavy metal salts (100 mg/L)

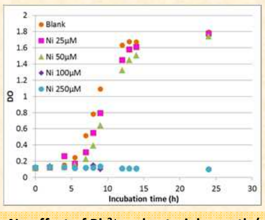


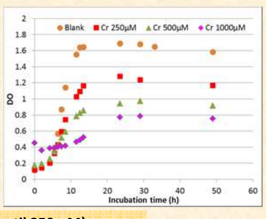


- Inhibitory effect: Ni << Zn = Cr   Activating effect: Cu > Pb
- Absence of metabolites (AMBA) in the presence of Cu^{2+}: Complexation and not biodegradation

- Effect of heavy metal salts on *Bacillus megaterium* Mes11 growth



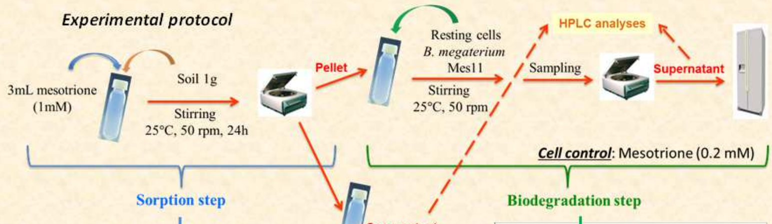


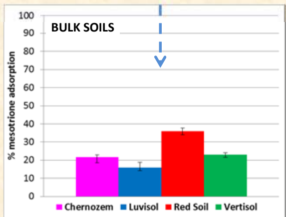


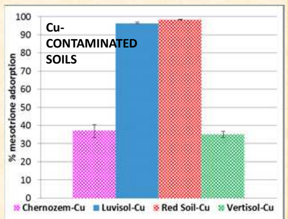
- No effect of Pb^{2+} on bacterial growth (until 250 µM)
- Strong toxicity of Cu^{2+} ions (Cu^{2+} >>> Ni^{2+} >> Cr^{3+} > Zn^{2+})

Impact of bulk and Cu-contaminated soils on mesotrione biodegradation

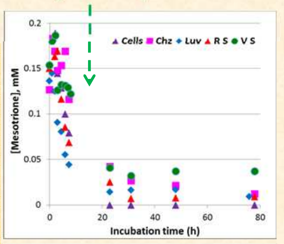
Experimental protocol

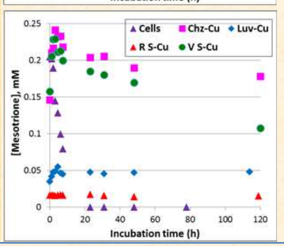






- Increased adsorption of mesotrione on Cu-contaminated soils
- No significant effect of the different soils on biodegradation except for Vertisol
  - Easy herbicide desorption
- Decrease of the mesotrione biodegradation kinetics in the presence of Cu-contaminated soils when not strongly adsorbed.
- No more biodegradation when mesotrione was no more available (Luvisol-Cu and Red Soil-Cu).





- Complexity of the system with numerous interactions involved between mesotrione – heavy metals – soils → **What about metabolite (AMBA) interactions?**
- Formation of a stoechiometric mesotrione-Cu^{2+} complex; Great increase of mesotrione sorption on Cu-contaminated soils
- Decrease of the mesotrione biodegradation kinetics in the presence of heavy metals (100 mg/L) in solution → **Heavy metal ad(ab)-sorption on biomass ?**
- Mesotrione less or no more bioavailable in Cu-contaminated soils – No biodegradation → **Mesotrione fate in real Cu-contaminated soil microcosms?**

REFERENCES: [1] I. Batisson et al., Isolation and characterization of mesotrione-degrading *Bacillus* sp. from soil, *Environ. Pollution*, **2009**, 157, 1195-1201.
[2] E. Dumas *et al.*, Fate and ecotoxicological impact of new generation herbicides from the triketone family: An overview to assess the environmental risks, *J. Hazard. Mater.*, **2017**, 325, 136-156.

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