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Short communication

Does antimony affect microbial respiration in Mediterranean soils? A microcosm experiment

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ABSTRACT

The aim of this study was to determine the effects of antimony on soil microbial respiration. Two Mediterranean calcareous soils were sampled: a contaminated soil close to an abandoned lead and silver smelter and a soil far from the pollution source and considered not to be contaminated. Two forms of antimony, antimony trioxide (Sb_2O_3) and potassium antimonyl tartrate trihydrate ($\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_{12}\cdot 3\text{H}_2\text{O}$), were tested at three concentrations (50, 500 and 5000 ppm) in controlled conditions under short- (3 days) and medium- (3 months) term incubation. Modifications in the substrate-induced respiration (SIR) were assessed by gas chromatography respirometric measurements. Results clearly showed that SIR was immediately and significantly more affected by Sb input in a non-contaminated soil than in a long-term contaminated soil, especially since the concentration was high and Sb was added to a more soluble and available form (tartrate instead of mineral oxide).

Antimony (Sb), a metalloid of the same periodic group as arsenic (As), is known to present toxicological properties, but its effect on the environment and on human health is little documented. World-wide production of Sb is considerably greater than of As, Sb having a wide range of uses (manufacture of semiconductors, diodes, flame-proof retardants, lead hardener, *etc.*) (Filella *et al.* 2002). As a consequence of its extensive use, many soils have been contaminated by this metalloid worldwide, through geogenic sources or anthropogenic pollution from activities such as mining, smelting, industry and agriculture (Douay *et al.* 2008).

Sb bioavailability and toxicity depends on speciation. Sb exists at 4 degrees of oxidation: -3 and 0 , which correspond to the metallic forms abundant in the soil of shooting ranges; $+3$ (or Sb (III)), the most toxic form and $+5$ (or Sb (V)), the most oxidised form (Wilson *et al.* 2010). Although Sb and As share some chemical properties, *e.g.* they both occur in soils as oxides, hydroxides and oxoanions, they have so far not been shown to act in the same way. In fact, the typology of the mechanisms of Sb transport and accumulation *via* adsorption/desorption processes remains relatively unknown (Wilson *et al.* 2010). Microbial communities can play a role in Sb mobility, and thus in its availability and toxicity, *via* oxidation, reduction and biomethylation mechanisms (Frohne *et al.* 2011). Surprisingly, to date very few attempts have been made to determine Sb effects on soil microbial communities. Thus, the toxicity of Sb for soil microorganisms is relatively unknown, but appears to

vary with the degree of Sb oxidation. Sb (III) appears to be ten times more toxic than Sb (V), while methylated Sb appears to be the least toxic (Wilson *et al.* 2010).

Here, therefore, we set out to determine using microcosms the effects of various Sb molecules and concentrations on soil microbial respiration. The Sb (III) oxidation degree was chosen, since this form is putatively the most toxic. Two poorly vegetated soils (calcosoil with surface colluvia) were sampled on the Mediterranean coast in the Marseilleveyre Massif, Marseilles (Bouches-du-Rhône, France). The first is a contaminated soil, herein called SC, located at the former Escalette metallurgical site, which extracted lead and silver from galena until the beginning of the last century, and close to an old horizontal smokestack running up a hillside to evacuate toxic fumes ($43^\circ 13'30''$ N, $5^\circ 20'54''$ E Southeast of Marseilles). This smelting spread smoke, aerial deposits and metallurgical slag around the buildings, resulting in heavy metal and metalloid contamination (mainly Pb, Zn, As, Sb, Cd and Cr). The second soil was sampled near the Sormiou creek ($43^\circ 12'47''$ N, $5^\circ 24'58''$ E), a site far from Escalette and thus considered non-contaminated (SNC). For each station, soil samples were collected from the surface layer (0–5 cm) in April 2011, sieved through a 2 mm mesh and stored at 4°C before use. The main physico-chemical characteristics of these soils, as well as their metal and metalloid contents, are given in Table 1.

Microcosms were prepared in plastic boxes $5\text{ cm} \times 5\text{ cm} \times 5\text{ cm}$, each containing 50 g of dry weight equivalent soil. Sb was supplied either as potassium antimonyl tartrate trihydrate ($\text{C}_8\text{H}_4\text{K}_2\text{O}_{12}\text{Sb}_{12}\cdot 3\text{H}_2\text{O}$, an organic highly soluble Sb form), herein called SbT, or as antimony trioxide Sb_2O_3 (a mineral poorly

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Table 1
Main physical and chemical characteristics of the studied soils.

	Soil	
	SNC	SC
Sand (%)	27.8	24.5
Silt (%)	34.1	56.7
Clay (%)	38.1	18.8
pH (H ₂ O)	7.88	7.37
C (g kg ⁻¹)	58.9	72.1
N (g kg ⁻¹)	3.45	4.4
C/N	17	16.4
CEC (cmol kg ⁻¹)	27.2	16
As (ppm)	17.7	6348
Cd (ppm)	ND	38.5
Pb (ppm)	87.4	38,306
Sb (ppm)	ND	1312
Zn (ppm)	103.6	11,112

soluble Sb form), herein called SbO. On the basis of cartographic field measurement (data not shown) and in order to cover a wide range of concentrations, three aqueous solutions of these two compounds, with Sb concentrations of 50 mg L⁻¹ (50 ppm), 500 mg L⁻¹ (500 ppm) and 5000 mg L⁻¹ (5000 ppm) were added to the soils to reach 60% of their water-holding capacities (WHC), yielding Sb concentrations of 6.8, 68 and 683 mg kg⁻¹ of dry soil (DW). Soil microcosms without Sb were used to perform controls: (i) soils

with distilled water added, termed C; (ii) to discriminate the Sb effect from the tartrate effect on SIR, soils with aqueous solutions of potassium sodium tartrate tetrahydrate (KNaC₄H₆O₆·4H₂O, >99%, Sigma-Aldrich) added, termed NaT, containing the same quantity of tartrate as SbT solutions. All microcosms were incubated in darkness at 20°C for 3 days (3d) or 3 months (3m). The lids of the boxes were perforated to allow the ventilation. The moisture content was maintained using sterile distilled water, the soil being weighted every 3 days. Five replicates were performed for each experiment and the design was completely randomised. Thus, for each incubation time (3d and 3m), a total of 50 microcosms were realised.

Substrate-induced respiration (SIR), *i.e.* CO₂ released when substrate is added (μg CO₂ h⁻¹ g⁻¹ DW), was measured according to Anderson and Domsch (1978). Briefly, ten grams of sub-samples at 60% of their WHC were weighed in 117 mL glass flasks and SIR was performed by adding 50 mg of powdered glucose to each soil sample (presumed saturating quantity; Anderson and Domsch 1978). Samples were pre-incubated for 90 min to reach a maximum rate of SIR. Then, after internal atmosphere replacement, flasks were closed hermetically and incubated for 60 min at 20°C. The CO₂ released in the flasks was measured by gas chromatography (GC, Chrompack CHROM 3 – CP 9001) and with a PorapakTM column containing 650 mL h⁻¹ helium circulating. Mann-Whitney's tests were used to assess whether the different treatments significantly influenced respiration.

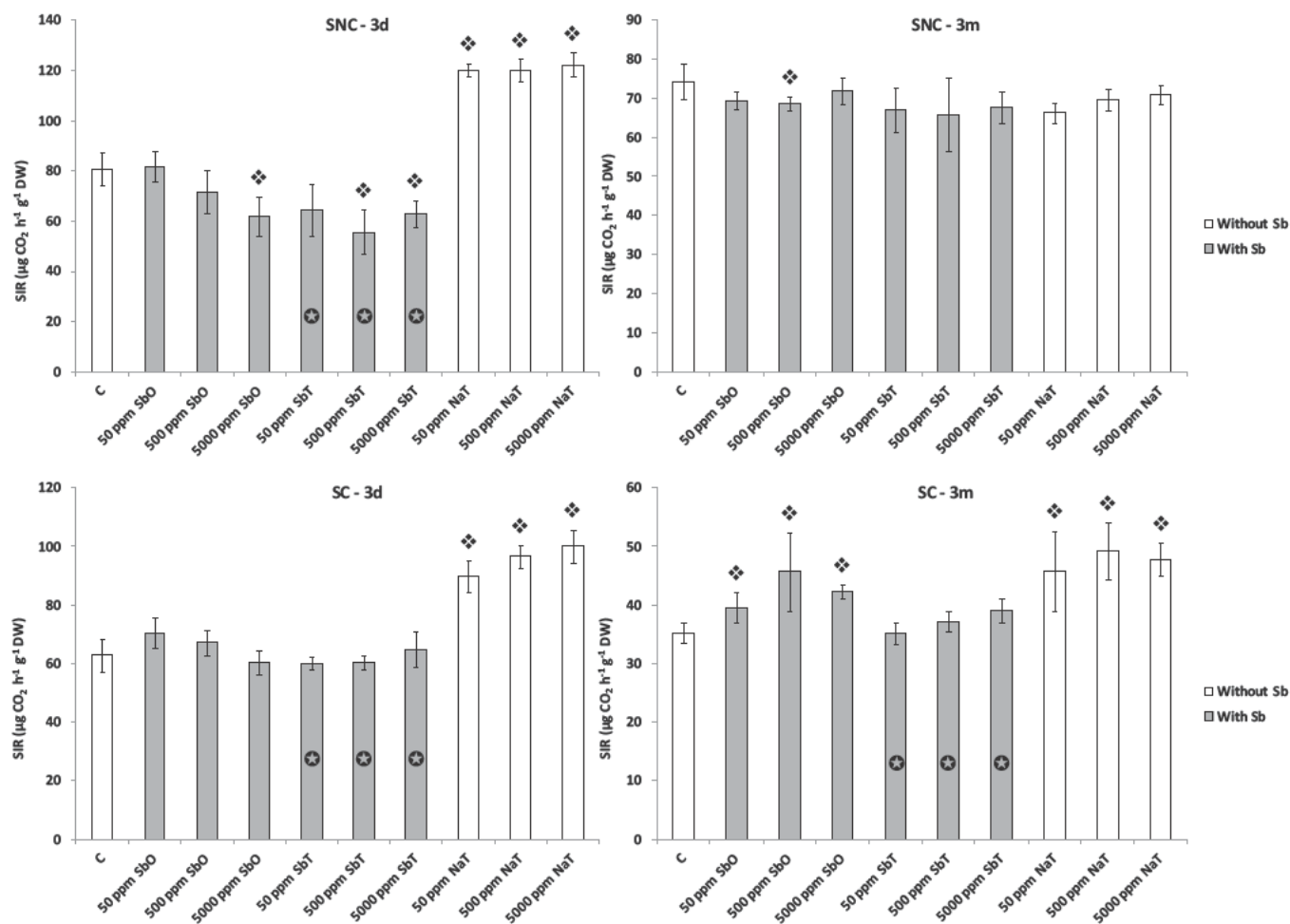


Fig. 1. Effects of antimony on SIR in a non-contaminated soil (SNC) and in a long-term contaminated soil (SC) after 3 days (3d) and 3 months (3m) incubation. Error bars represent the standard error of mean of five replicates (n = 5). Significant differences from non-treated soils (C) at $p < 0.05$ (♦) and between SbT and NaT treated soil at $p < 0.05$ (⊕) are shown (Mann-Whitney test). SbO: antimony trioxide (Sb₂O₃); SbT: potassium antimonyl tartrate trihydrate (C₈H₄K₂O₁₂Sb₁₂·3H₂O); NaT: potassium sodium tartrate tetrahydrate (KNaC₄H₆O₆·4H₂O).

After 3-day incubation (Fig. 1), SIR was more significantly affected by Sb input in a non-contaminated soil than in a long-term contaminated soil, especially since the concentration was high and Sb was added to a more soluble and available form (tartrate instead of mineral oxide). In both soils and for both Sb forms, SIR decrease can be explained by several physiological disturbances occurring at the cell level. Sb toxicity is known to induce oxidative stress resulting in lipid peroxidation, increased membrane permeability and cell lysis by loss of cytosolic constituents. Moreover, Sb has the ability to substitute to P in biological reactions and it can also more directly affect the respiration of soil microorganisms, since it can combine with certain thiol groups of respiratory enzymes (e.g. dehydrogenases) and thus inhibit cellular energy supply (Tirmenstein et al. 1995; Shtangeeva et al. 2011). Adding the poorly soluble form of Sb, Sb_2O_3 , resulted, over a short-term incubation (3d), in lower SIR activities in the non-contaminated soil. This inhibition increased with the Sb dose applied, but the relation between the two variables was not proportional. Such results are consistent with those of Oorts et al. (2008) who reported that: (i) the dissolution rate of Sb increased almost three-fold when the applied dose of Sb_2O_3 was increased from 50 to 10,000 mg Sb kg⁻¹; (ii) inhibition of soil respiration processes (i.e. nitrifying aerobic respiration) also occurred only at the higher dose.

After a 3-month incubation, positive effects of Sb_2O_3 occurred on SIR and remained for us unexplained. Despite this atypical response, the inhibitive effects of both Sb_2O_3 and Sb-tartrate observed at 3-d were, in most cases, nil or greatly reduced at 3-m. This could be explained by the establishment of resistance responses by microbial communities such as ATPase pumps, chemo-osmotic antiports, oxydoreduction mechanisms and intracellular or membranar retention (Rosen 2002). Surprisingly, SIR was still decreased by SbT with regards to NaT in the contaminated soil, whereas it was not in the non-contaminated. Since the contaminated soil had far lower clay content and CEC, another reason for the pattern observed may be due to lower Sb immobilisation on soil particles resulting in higher availability and toxicity towards microorganisms (Flynn et al. 2003). Despite it was not possible for

us to quantify the different Sb forms, a further explanation for the decrease in toxic effects observed during our experiments could be a modification in the speciation of Sb. Sb (III) is known to be instable in oxic soils, and is rapidly oxidised by Fe–Mn-oxihydroxides and humic acids in its Sb (V) form, which is less toxic than the Sb (III) form (Oorts et al. 2008).

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