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Growth parameters influencing uptake of chlordecone by *Miscanthus* species

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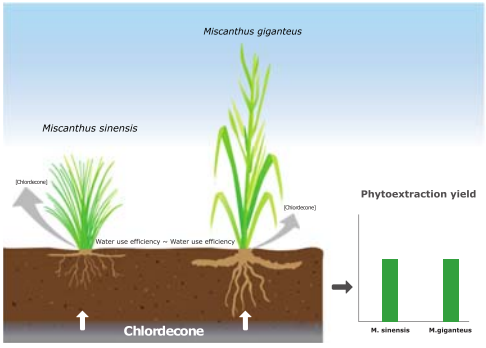
HIGHLIGHTS

- Chlordecone uptake was compared in *Miscanthus* sp. with contrasted development and morphology.
- Perennial rhizomes do not promote chlordecone transfer to aboveground plant parts.
- Plant biomass is not sufficient to assess the efficiency of phytoextraction.
- Concentrations in aboveground plant parts decrease in successive harvests.

GRAPHICAL ABSTRACT



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ABSTRACT

Because of its high persistence in soils, $t_{1/2} = 30$ years, chlordecone (CLD) was classified as a persistent organic pollutant (POP) by the Stockholm Convention in 2009.The distribution of CLD over time has been heterogeneous, ranging from banana plantations to watersheds, and contaminating all environmental compartments. The aims of this study were to (i) evaluate the potential of *Miscanthus* species to extract chlordecone from contaminated soils, (ii) identify the growth parameters that influence the transfer of CLD from the soil to aboveground plant parts. CLD uptake was investigated in two species of *Miscanthus*, C4 plants adapted to tropical climates. *M. sinensis* and *M.* × *giganteus* were transplanted in a soil spiked with [^{14}C]CLD at environmental concentrations (1 mg kg^{-1}) under controlled conditions. Root-shoot transfer of CLD was compared in the two species after two growing periods (2 then 6 months) after transplantation. CLD was found in all plant organs, roots, rhizomes, stems, leaves, and even flower spikes. The highest concentration of CLD was in the roots, 5398 ± 1636 (*M.* × *giganteus*) and 14842 ± 3210 ng g^{-1} DW (*M. sinensis*), whereas the concentration in shoots was lower, 152 ± 28 (*M.* × *giganteus*) and 266 ± 70 ng g^{-1} DW (*M. sinensis*) in soil contaminated at 1 mg kg^{-1}. CLD translocation led to an acropetal gradient from the bottom to the top of the plants. CLD concentrations were also monitored over two complete growing periods (10 months) in *M. sinensis* grown in 8.05 mg kg^{-1} CLD contaminated soils. Concentrations decreased in *M. sinensis* shoots after the second growth period due to the increase in organic matters in the vicinity of the roots. Results showed that, owing to their respective biomass production, the two species were equally efficient at phytoextraction of CLD.

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1. Introduction

Chlordecone (CLD) is an organochlorine pesticide that was mainly used in the French West Indies to try and control the black banana weevil (*Cosmopolites sordidus*). CLD was spread in Guadeloupe and Martinique from 1972 to 1993. It was applied in the form of a powder, directly on the soil at the base of the banana stem. Two formulations were used, Kepone from 1972 to 1976 and Curlone from 1981 to 1993, both with 5% active ingredient. It is estimated that 3 kg CLD per hectare and per year were applied over a period of 20 years. The use of CLD has been banned in the French West Indies since 1993. Catchment areas and old banana plantations are currently contaminated with concentrations ranging from 0.2 to 50 mg kg^{-1} of soil. The contaminated area accounts for 15% and 19% of the Utilized Agricultural Land (UAL) in Guadeloupe and Martinique, respectively. CLD was also used in banana plantations in Africa and as Kelevan (CLD precursor) in Eastern Europe against the Colorado potato beetle (Epstein, 1978).

CLD is persistent in soils (Cabidoche et al., 2006) and was classified as a POP in 2009 because of its low polarity conferred by the high chlorination (Cl_{10}) and its lipophilic log K_{ow} values that range from 4.50 (Howard, 1991) to 5.40 (UNEP, 2006). Contamination represents both environmental and health risks (Cabidoche et al., 2009; Multigner et al., 2010).

Because of its lipophilic nature, CLD has a strong affinity for soil organic matter. It is transferred to aquatic environments either by surface runoff in downstream watersheds or by leaching into the groundwater (Levillain et al., 2012). Contamination and bio-magnification of CLD in aquatic trophic chains have both been demonstrated (Coat et al., 2011). Cabidoche et al. (2006) showed that sugarcane accumulated CLD in stalks when it grew on contaminated soils. Recently, Lorber-Pascal et al. (2016) showed translocation of CLD to maize shoots despite its high rate of adsorption on root surfaces. Likewise, the edible root pulp or flesh of some cucurbits is contaminated by CLD.

Owing to health and environmental risks, finding a solution to reduce CLD contamination of soils is crucial for the population of the French West Indies. Highly polluted soils are arable lands, former banana plantations, and the total area to be treated is 11,500 ha. Consequently, conventional ways of remediating the pollution such as excavation or chemical techniques are not valid. Bioremediation, phytoremediation or both are thus more likely and less expensive alternatives. For some years, researchers have been focusing on the capacity of soil microorganisms to degrade CLD. Despite high expectations for the process, the results of this method are still not very encouraging (Merlin et al., 2014). In this context, phytotechnologies are a viable alternative (Kang, 2014). Phytoremediation of contaminated soils by organic compounds is mainly based on plant ability to favor microorganism activity on roots (rhizodegradation) (Meggo and Schnoor, 2013). Plants can also take up the compounds and store them (phytoextraction) or metabolize them (phytodegradation) (Pilon-Smits and Freeman, 2006; Schwitzguébel, 2017). Plant uptake of organic compounds and their mobility within plant after root absorption is driven by their lipophily (Briggs et al., 1982). CLD is transported through the water stream in plants (Lorber-Pascal et al., 2016). The main mechanism of phytoextraction is hypothesized to be evapotranspiration. In addition to trees, the plants currently used for phytoremediation are Gramineae, in particular the common reed (*Phragmites australis*) (Guittonny-Philippe et al., 2015) and bamboo sp. (*Bambuseae*) (Piouceau et al., 2014). Gramineae are suitable candidates for phytoremediation, because of their short generation time and the large quantity of biomass they produce. *Miscanthus* is a woody perennial grass that can grow in temperate and tropical climates. This grass has several advantages for phytoremediation. It is able to grow on highly contaminated soils (Wanat et al., 2013). Moreover, *Miscanthus* roots can produce exudates that promote the biostimulation of PAH degraders (Técher et al., 2011, 2012a) and improve the agronomic quality of the soil (Técher et al., 2012b). *Miscanthus* is thus a good candidate for revegetation and soil restoration of industrial wasteland (Técher et al., 2012c.). In addition, *Miscanthus*' high biomass can be used in different ways, mainly as alternative biofuel or as building material (Chou, 2009; Pidlisnyuk et al., 2014). The question is whether a high biomass-producing plant like *Miscanthus* is a good candidate for phytoextraction of CLD.

The objective of this study was to evaluate the capacity of *Miscanthus* species to take up CLD from a freshly contaminated soil and to highlight the growth parameters that influence its translocation up to its aboveground plant parts. Two species were compared, differing in their shoot development and rhizome size, *Miscanthus* × *giganteus*, which biomass is currently used to produce energy and one of its parents, *Miscanthus sinensis*. *Miscanthus* has a perennial rhizome that remains in the soil from one year to the next for 15 years. *Miscanthus* sp. was chosen to determine if the presence of rhizomes has an influence on the translocation of CLD, as suspected in sugarcane.

2. Materials and methods

2.1. Chemicals

[^{14}C]CLD (specific activity = 1.443 GBq $mmol^{-1}$, radiochemical purity >97% as determined by radio-RP-HPLC analysis) was purchased from Moravek (Brea, CA). Analytical standard CLD (Fluka 45379, PESTANAL®) was purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France) and solvents from Scharlau Chemie S. A. (Barcelona, Spain). Unless otherwise specified, all other chemicals were of analytical grade.

2.2. Plant growth conditions

For all experiments, *Miscanthus* plants were grown in a temperature-controlled room with a 16 h photoperiod (27 °C day and 20 °C night). They were watered second day with tap water and weekly with a nutrient solution (Coic and Lesaint, 1973).

2.3. Time-dependent uptake of [^{14}C]CLD by Miscanthus sinensis

2.3.1. Soil and experimental design

Miscanthus sinensis plants (cv. Fendengo, Les espaces verts du Languedoc, Toulouse, France) were grown in a metal container, 0.9 m × 0.9 m × 0.15 m, containing 125 kg of soil (standard soil type no 2.4, pH 7.2, sieved to 2 mm, Speyer, Germany) contaminated with [^{14}C]CLD. [^{14}C]CLD was first used as an acetone/water solution (85:15, v/v) to spike 2 kg of soil before being progressively added to the whole soil volume, which was then homogenized to obtain 8.05 mg kg^{-1}, 131,792 Bq kg^{-1}. *Miscanthus* rhizomes (49) were transplanted in squares, 7 columns (1–7) and 7 rows (A–G).

2.3.2. Harvesting plan and sample preparation

The harvesting plan was a random sampling using a random Latin square design. Plants located along the edges were not harvested. Three *Miscanthus* plants were harvested monthly for 4 months, then at 6 and 10 months during the first growing period. At the beginning of the second growing period (after a two-month break between the two cycles), aboveground plant parts were cut and the plants were harvested 10 months later. The underground parts, rhizomes and roots, were separated from the aboveground plant parts, stems and leaves. The rhizomes and roots were rinsed several times under running water. Each organ was analyzed separately. Plant samples were dried at 60 °C for 48 h and then ground in an MM 400 mixer mill (Retsch GmbH, Haan, Germany). One aliquot was used to quantify radioactivity. Between the two growing periods, all the shoots of the plants that were not harvested were cut off before flowering to induce the growth of new stems.