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ORIGINAL PAPER

Dynamics of the toxic cyanobacterial microcystin-leucine-arginine peptide in agricultural soil

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Abstract Microcystin-leucine-arginine (MC-LR) is a cyclic heptapeptide hepatotoxin produced by cyanobacteria such as *Microcystis aeruginosa*. Being highly toxic, this compound is a threat to water quality, agriculture, and human and animal health. In particular, MC-LR has been frequently detected at high concentrations in surface waters. So far, the fate of MC-LR in soils is unknown. Here, we studied degradation and soil–plant transfer of ^{14}C-radiolabelled MC-LR in an artificial system of agricultural soil and tomato seedlings. ^{14}C-MC-LR was dissolved in water and applied by soil irrigation, one or two times with an interval of 28 days. Results show that the $^{14}CO_2$ from the degradation of ^{14}C-MC-LR amounted to 11 % of total ^{14}C initial input; 74–80 % of ^{14}C-MC-LR occurred in extractible fractions analysed by HPLC. Less than 14 % of ^{14}C-MC-LR was adsorbed on soil particles. Overall, our findings evidence for the first time a high risk of toxin leaching from the soil toward groundwater.

Keywords Cyanotoxins · Microcystin-LR · Soil · Persistence · Mineralization · Plant transfer

Introduction

The occurrence of harmful cyanobacterial blooms in surface waters is often accompanied by the production of a variety of cyanotoxins, which include potent hepatotoxins, neurotoxins and dermatotoxins that represent a growing problem for fisheries, agriculture and public health. According to Ettoumi et al. (2011), studies in several countries revealed microcystins production in 50–75 % of cyanobacterial samples investigated, where more than 80 variants already reported (Sivonen and Jones 1999; del Campo and Ouahid 2010). Microcystins are cyclic peptides that inhibit enzymes called protein phosphatases both in vertebrate and in vegetable cells, which are involved in the regulation of many important cellular processes (MacKintosh et al. 1995; Kurki-Helasmo and Meriluoto 1998; Hastie et al. 2005). Among these hepatotoxins, the congener microcystin-LR (MC-LR) is generally recognized as being the most studied due to its high toxicity, frequent detection, and its concentrations in surface waters often exceed the World Health Organization advisory level of 1 μg L^{-1} (see in Corbel et al. 2014).

In surface waters used as irrigation source, total microcystin concentrations of a few dozen up to a few hundred of μg L^{-1} have been reported in several countries throughout the word (Corbel et al. 2014). Consequently, the use of these contaminated waters for spray irrigation of terrestrial plants, including food crop plants, presents a harmful effect both on growth and development of plants and on soil organisms. In addition, microcystins present also potential health hazards through accumulation in plants (Corbel et al. 2014) and infiltration in the groundwater (Eynard et al. 2000). Questions, therefore, arise about their persistence when reach the soil ecosystem. Microcystins are water-soluble molecules, and their cyclic structure provides them a high chemical stability. Nevertheless, numerous studies reported that these cyanotoxins can be removed in aquatic ecosystems according to various process such as photochemical degradation by UV (Tsuji

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et al. 1994), adsorption in particles in suspension or onto sediments (Wörmer et al. 2011) and biodegradation by several bacteria species (Chen et al. 2008, 2010). However, the fate of these toxins in agriculture soil ecosystems is particularly scarce. During soil irrigation practice, one part of microcystins may be degraded rapidly by sunlight or by some species of soil bacteria (Bourne et al. 2001; Manage et al. 2009) but another part could persist. Their half-life in soils was estimated between 6 and 17.8 days (Chen et al. 2006b). Morris et al. (2000) and Miller et al. (2001) showed that the clay and organic matter content in soils played a key role in the microcystins adsorption. Nevertheless, these works were conducted in bath experiments where the conditions were not similar to the environmental conditions. On the other hand, it seems that the adsorption of microcystins onto the soil was low. Consequently, the lixiviation of microcystins and the contamination of groundwater by these toxins originated from surface water were advanced by Eynard et al. (2000). In addition, the microcystins could be strongly available for soil organisms and plants.

The objective of the present work was to examine the fate of microcystins in an agriculture soil using the variant microcystin-LR radiolabeled with carbon 14 (^{14}C-MC-LR) as a representative example of these toxins family. This approach will contribute to an enhancement of our understanding both on the availability of this toxin in an agricultural soil and its potential transfer to plants and on the risk of its infiltration in the groundwater. It was the first study on the fate of MC-LR in agricultural soil after irrigation with contaminated water.

Materials and methods

Chemicals and reagents

Chemicals and solvents were of analytical grade. All chemicals, including the standard of microcystin-leucine-arginine (MC-LR), were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France), and solvents from Carlo-Erba (Val de Reuil, France). Labelled sodium bicarbonate, ^{14}C-$NaHCO_3$ (specific activity 310 MBq mmol^{-1}) was supplied from American Radiolabeled Chemicals (Isobio, Fleurus, Belgium).

Soil characteristics

The topsoil from the experimental meadow site of INRA, Pierre-Plate (Versailles, France), was collected in August 2012. It was a silty sand soil (Luvic Cambisol/Cambisol, IUSS working Group WRB 2006) comprising 76 % sand, 13 % silt and 11 % clay. Clay consisted mainly in illite/smectite. Its content in organic matter was 37.8 ‰, and its pH water was 5.6. Its water-holding capacity was 35.5 %. The soil was homogenized, air-dried, sieved at 2 mm and stored at 20 °C.

Culture of *Microcystis aeruginosa* for microcystin-leucin-arginine (^{14}C-MC-LR) biosynthesis

The *Microcystis aeruginosa* strain PCC7820 (Pasteur Institute, Paris, France) was used to synthesize the ^{14}C-radiolabelled MC-LR. The strain was grown axenically at 25 °C with an incident light intensity of 5–10 µmol of photons m^{-2} s^{-1} (16:8 h light/dark cycle) as previously reported by Gupta et al. (2001). Batch culture in 1-L sealed flask contained 500 mL of BG_{11} liquid medium supplemented with 2 mM $NaNO_3$ and 5 mM unlabeled $NaHCO_3$. The culture was continuously bubbled with sterile air at a flow rate of 0.23 L min^{-1}, and the outlet of the flask was connected to a CO_2 trap (see below). Three additions of an aqueous solution of 0.62 MBq ^{14}C-$NaHCO_3$ were performed after 7, 10 and 13 days of culture, according to modified protocols from Hyenstrand et al. (2003) and Nybom et al. (2012). The culture was stopped 4 days later, corresponding to a 10-day period of growth in presence of labelled bicarbonate.

Mass-balance analysis of radioactivity in the cultures and extraction of labelled cyanotoxins

The mineralization of ^{14}C-$NaHCO_3$ was monitored by continuous trapping of $^{14}CO_2$ in 100 mL of 1 N NaOH solution. The solutions were replaced every 2–3 days, and the amounts of dissolved $^{14}CO_2$ were determined by liquid scintillation counting using Optiphase Hisafe 2 (Perkin Elmer, Courtaboeuf, France) as liquid scintillation cocktail. At the end of the culture, cyanobacteria cells were harvested by centrifugation (4,000*g*, 10 min) and the radioactivity remaining in the culture medium was determined by liquid scintillation counting. Fresh cells of cyanobacteria were homogenized with 24 mL of 75 % (v/v) aqueous methanol (MeOH). The homogenate was sonicated for 3 min in an ultrasonic bath, followed by centrifugation at 4,000*g* for 10 min. The supernatant was retained, and the residue re-extracted two times as before. Combined supernatants formed the cyanobacterial extract containing microcystins, stored at −20 °C prior to further analysis. Residual radioactivity in the cyanobacteria cells was determined by combustion of dry aliquots with a Biological Oxidizer OX 700 (Zinsser Analytic, Frankfurt, Germany). The $^{14}CO_2$ produced was trapped and measured with Oxysolve C400 (Zinsser analytic) as liquid scintillation cocktail. Several successive cultures were repeated to obtain sufficient amounts of ^{14}C-MC-LR for soil experiments.

Purification of radiolabelled microcystin-leucine-arginine (^{14}C-MC-LR)

The cyanobacterial extract containing microcystins was diluted with MilliQ water to obtain a final concentration of 20 % aqueous MeOH (v/v). A Sep-Pack® C18 cartridge (Waters Corp., Guyancourt, France) was conditioned with 20 mL of pure MeOH and 20 mL of MilliQ water. The crude extract of cyanobacteria was then applied onto the conditioned cartridge at 1 mL min^{-1} according to Ramanan et al. (2000). The microcystins were then eluted using 10 mL of MeOH acidified with 0.1 % (v/v) trifluoroacetic acid. The ^{14}C-MC-LR congener was purified by preparative Liquid Chromatography with a Kromasil® C18 column (250 × 10.5 mm i.d., 5 µm) supplied from Merck (Interchim, Montluçon, France). The mobile phase comprised water (solvent A) and acetonitrile (solvent B), each acidified with 0.05 % trifluoroacetic acid, at the flow rate of 1 mL min^{-1}. The gradient consisted of 10 min at 80 % solvent A, then 60 % solvent A during 5 min, 20 % solvent A during 5 min and finally 100 % solvent B during 10 min. The injection volume was 200 µL. The A_{238} of the column eluate was monitored with a UV–Vis detector (Shimadzu SDD-10A). Under these chromatographic conditions, the retention time of MC-LR is 10.0 min. One millilitre fractions were also collected, and their radioactivity determined by liquid scintillation counting. The fractions containing the ^{14}C-MC-LR congener were combined, and acetonitrile of the solution was evaporated under nitrogen flux. The aqueous solution was frozen (−20 °C) before further use.

Determination of specific activity of aqueous solution of radiolabelled microcystin-leucine-arginine (^{14}C-MC-LR)

Different concentrations of aqueous MC-LR standard were injected in liquid chromatography. The calibration curve allowed determining the concentration of ^{14}C-MC-LR in the aqueous solution obtained from the cyanobacterial culture. In parallel, the radioactivity of the solution was obtained by liquid scintillation counting. The specific activity of the purified ^{14}C-MC-LR was determined to be 577 kBq mmol^{-1} (see the "Results and discussion" section).

Experimental design of soil experiments

Three days before the beginning of the experiments, 24 g of air-dried soil was disposed in glass columns (3.5 cm i.d.) and moistened to 60 % of water-holding capacity. Five soil columns have been prepared for our incubations. The first one (referred as the control) was not spiked with the ^{14}C-MC-LR, but was submitted to below-mentioned protocols and analyses. It allowed us to obtain the background values used for calculations. The next three columns were spiked with 3.2 mL of aqueous solution of ^{14}C-MC-LR (0.5 kBq column^{-1}) that increase the soil water-holding capacity to 100 % and incubated during 1 h (referred as T0), 28 (T28) or 56 days (T56). The total amount of toxin added to the soil corresponded to 862 µg and ensured a final concentration of 36 mg kg^{-1} dry soil in these columns. The last column (entitled "two treatments") received an input of ^{14}C-MC-LR at the beginning of the experiment and was submitted to a second similar treatment after 28 days and was incubated during a further 28-day period. The total amount of toxin added to the soil corresponded to 1,724 µg of MC-LR and 72 mg kg^{-1} dry soil. Its final incubation time was 56 days. It allowed the observation of the effect of repeated exposure by comparison with other columns submitted to single treatment. All the columns were incubated in the dark at 25 °C in one-litre sealed flasks in the presence of vials containing 5 mL of 1 N NaOH and 10 mL of water, during 28 or 56 days. Every 7 days, the solution of NaOH was changed and the radioactivity was determined by liquid scintillation counting. At the end of the experiments, the columns were frozen (−20 °C) before analysis.

Mass-balance analysis of radioactivity in the soil columns

The mineralization of ^{14}C-MC-LR in soil columns was monitored by trapping the evolved $^{14}CO_2$ in 5 mL 1 N NaOH in vials. The solutions were replaced every 7 days. Leachates were collected at the end of each incubation after watering the columns with 25 mL of 0.01 M $CaCl_2$ (OECD 2004). The leachates corresponded to 20 mm rainwater. These fractions were referred as the very available/mobile fractions of MC-LR in the soil. Then, 10-g fractions of wet soils from the columns were then disposed in centrifuge tubes for complete extraction of the MC-LR. A 15-mL solution of 0.1 M of ethylene diamine tetra acid (EDTA) a chelating agent and 0.1 M $Na_4P_2O_7$, acidified with 2 mM trifluoroacetic acid, was added in the tubes according to Chen et al. (2006a). The tubes were shaken (700 rpm, 10 min), then sonicated for 10 min in an ultrasonic bath at 0 °C and finally centrifuged at 4,000 g for 10 min. Two other extractions were performed in the same conditions, and the supernatants from each column were pooled. Then, final extractions were performed using the same protocol, with aqueous MeOH solutions (20, 50 and 100 % (v/v)). These fractions were referred as the adsorbed fractions of MC-LR in the soil. The radioactivity in all liquid fractions was quantified by liquid scintillation. Residual radioactivity in the extracted soils was determined

by combustion of dry aliquots with the Biological Oxidizer OX 700 as described above. These fractions were referred as the bound MC-LR in the soil.

Purification and analysis of aqueous soil extracts

Aqueous soil extracts ($CaCl_2$, EDTA+$Na_4P_2O_7$ solutions) were purified using solid-phase extraction C18 cartridge, 500 mg (Bond Elut C18, Agilent Technologies). The cartridges were previously conditioned with 5 mL MeOH and 5 mL MilliQ water. The aqueous extracts were then loaded on the cartridges at the flow rate of 1 mL min^{-1}. The radioactive compounds were eluted with 3 mL MeOH acidified with 0.1 % trifluoroacetic acid. The eluates were evaporated to dryness under nitrogen flux and solubilized in 500 µL aqueous 50 % acetonitrile (v/v). The extracts were then frozen (−20 °C) before analysis.

For analysis, the extracts were diluted to 10 mL with acidified water and concentrated online onto a C18 guard column MCH-10 (3 cm × 4 mm i.d., Varian, Les Ulis, France) connected to a ten-port valve, at a flow rate of 1 ml min^{-1} with an isocratic pump, according to Mougin et al. (1996). The elution of labelled compounds was then achieved onto the analytical Kromasil® C18 column (250 × 4.6 mm i.d., 5 µm) supplied from Macheray-Nagel (Interchim, Montluçon, France). The mobile phase of the liquid chromatography system comprised water (solvent A) and acetonitrile (solvent B), each acidified with 0.05 % trifluoroacetic acid, at the flow rate of 1 mL min^{-1}. The gradient consisted of 8 min at 70 % solvent A, then a linear increase to 100 % solvent B during 5 min and a stationary of 100 % solvent B during 5 min. The injection volume was 20 µL. The A_{238} of the column eluate was monitored, one-mL fractions were also collected, and their radioactivity determined by liquid scintillation counting. Under these chromatographic conditions, the retention time of MC-LR is 14.3 min.

The radioactivity trapped in the columns was determined by combustion of silica with the Biological Oxidizer OX 700 as described above.

Transfer of microcystin-leucine-arginine (^{14}C-MC-LR) from soil to tomato seedlings

Approximately 15 g of fresh soil fractions from the three columns exposed to ^{14}C-MC-LR during 28 or 56 days was mixed with 15 g unexposed soil and disposed in dishes. Three seeds of tomato *Solanum lycopersicum* variety MicroTom obtained from INRA (Avignon, France) were allowed to germinate in non-contaminated sand and sowed after 2 weeks of growth in each dish. Soil moisture was maintained in dishes at 100 % of water-holding capacity, and the dishes were then incubated in hermetic jars with water vial to regulate evaporation. The three jars were disposed at 25/20 °C (light/dark cycle). After 2 weeks of incubation, seedlings were harvested and weighed, and their content in ^{14}C was determined by combustion of dry aliquots with the Biological Oxidizer OX 700 as described above.

Results and discussion

Biosynthesis of radiolabelled microcystin-leucine-arginine (^{14}C-MC-LR)

The synthesis of the radiolabelled microcystin-LR (^{14}C-MC-LR), a toxic cyanobacterial heptapeptide, was obtained from a culture of *M. aeruginosa* grown during 10 days in the presence of ^{14}C-$NaHCO_3$. Typically, the ^{14}C mineralized amounted to 76 % of the initial radioactivity at the end of the cultures. Another part of ^{14}C-$NaHCO_3$ remained in the culture medium of cyanobacteria. It represented 9 % of the initial radioactivity and corresponded mainly to ^{14}C-$NaHCO_3$. Approximately 12 % of the initial radioactivity was retained in the cyanobacteria cells. Finally, only 3 % of initial ^{14}C were converted to ^{14}C-MC-LR. The primary metabolism of cyanobacteria that uses carbon as nutrient explained the high mineralization of carbon and the accumulation of ^{14}C in the cyanobacteria cells. The synthesis of MCs resulting from the secondary metabolism of cyanobacteria leveraged lower amounts ^{14}C-$NaHCO_3$. After purification, the specific activity of ^{14}C-MC-LR was determined to be 0.58 Bq μg^{-1}, corresponding to 577 kBq $mmol^{-1}$. This value is in accordance with the recently study by Nybom et al. (2012) who also used ^{14}C-$NaHCO_3$ as precursor. Nevertheless, these specific activity values were lower than those reported by Craig et al. (1996), Pflugmacher et al. (1998) and Hyenstrand et al. (2003) which were 33.00, 2.96 and 18.30 Bq μg^{-1} MC-LR using the same precursor, but different cyanobacterial strains. It is likely that the efficiency of ^{14}C-MC-LR production depends on both the strains and their culture conditions used.

Mineralization of radiolabelled microcystin-leucine-arginine (^{14}C-MC-LR) in soil

The mineralization of ^{14}C-MC-LR was monitored in the soil columns (Fig. 1). No $^{14}CO_2$ was evolved from the non-spiked column. By contrast, 7 days after soil spiking, mineralization amounted to the mean value of 8 % of the initial labelled carbon in the columns submitted to a single treatment with ^{14}C-MC-LR. Then the yield curve decreased, and 11 % of the initial carbon was mineralized after 28 days of incubation (Fig. 1a). A similar pattern was observed in the column submitted to a second treatment with ^{14}C-MC-LR.

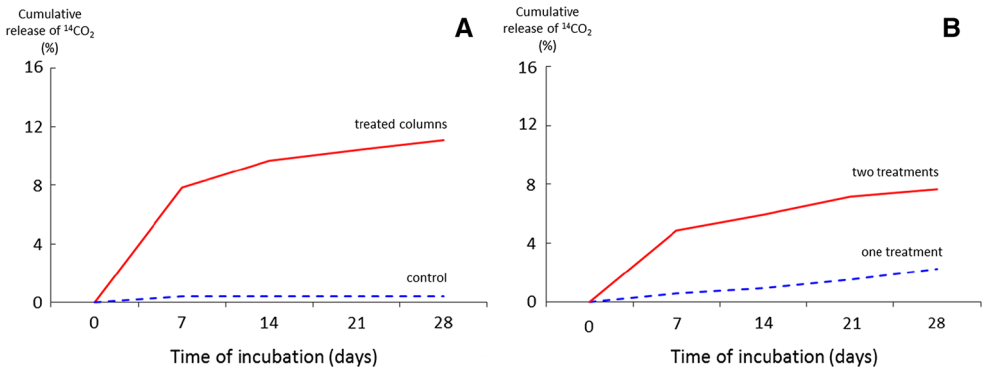

Fig. 1 Mineralization of radiolabelled microcystin-leucine-arginine (^{14}C-MC-LR) in soil columns receiving a single treatment with water containing labelled cyanotoxin (**A**) and in the column submitted to two successive treatments with the labelled cyanotoxin (**B**). Note the strong mineralization 7 days after soil exposition and then the decrease in it

Nevertheless, only 5 % of the carbon was mineralized after 7 days and 8 % after 28 days (Fig. 1b). Our results demonstrated for the first time the capacity of soil microorganisms to partly mineralize MCs. By comparison, Cousins et al. (1996) observed in laboratory experiment a rapid degradation of MC-LR contained in water in less than 7 days after exposition. In freshwater, numerous species of bacteria were able to promote MCs degradation such as *Sphingomonas* sp. strain ACM-3962, *Paucibacter toxinivorans* and *Stenotrophomonas* sp. (Jones et al. 1994; Rapala et al. 2005; Chen et al. 2010). In addition, soil bacteria could also degrade MCs as *Arthrobacter* sp. *Brevibacterium* sp. or *Rhodococus* sp. (Manage et al. 2009).

The moderate mineralization of ^{14}C-MC-LR observed in the present study could be explained through many reasons. First of all, one part of the ^{14}C-MC-LR was easy to breakdown 7 days after exposition, and then, the degradation proceeded slowly. These rates of mineralization after 28 or 56 days were low, but they could be explained by the high concentrations of MCs used for soil irrigation: 0.1 µg µL^{-1}. MCs were known to be involved in the regulation of physiological processes by dephosphorylation of regulatory proteins (Corbel et al. 2014), and soil microorganisms could be affected by the toxicity of MCs. In addition, a study of Valdor and Aboal (2007) showed an inhibition of growth of *Streptoverticillum* sp. and *Escherichia coli*, after culture on induction medium with MCs concentrations of 25 and 50 µg mL^{-1}. By contrast, Corbel et al. (in revision) observed a stimulation of bacterial nitrifying activity in soil after daily exposition with concentrations comprised between 5 and 50 µg eq. MC-LR L^{-1}, during 14 days. In addition, soil global enzymatic activities (phosphatase, glucosidase and arylsulfatase) were not affected by treatments with MC-LR at 36 and 72 mg kg^{-1} dry soil by comparison with values obtained in the non-spiked controls (data not shown) They amounted to values typically noticed in French agricultural soils and

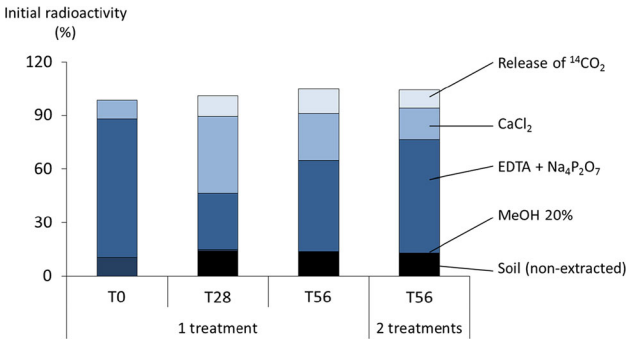

Fig. 2 Mass-balance analysis of the radioactivity in the four soil columns submitted to treatments with water containing labelled cyanotoxin. Fractions were obtained at the beginning of the incubations, and after 28 and 56 days of incubation. Note the evolution of radiolabelled microcystin-leucine-arginine (^{14}C-MC-LR) adsorption onto soil according to the time of incubation

attested of a significant microbial activities in our columns. In addition, even modified after treatment with cyanobacterial extracts, soil microbial communities may maintain a significant metabolic activity, as observed in freshwater communities (Giaramida et al. 2013). The ^{14}C-MC-LR was not fully mineralized in the soil used for our experiments and may accumulate following successive inputs. Furthermore, the composition of soil could modify both the fate and the availability of MCs for the degrading organisms.

Fate of microcystin-leucine-arginine (^{14}C-MC-LR) in the soil columns

The mass-balance analysis of labelled carbon was performed in the columns treated with ^{14}C-MC-LR at the beginning of the experiment and then after 28 and 56 days of incubation (Fig. 2). One hour after soil spiking, labelled carbon in the columns was mainly associated with the

EDTA extract (77.6 % of initial labelled carbon applied), and only 10.6 and 10.4 % of labelled carbon from initial ^{14}C-MC-LR were quantified in $CaCl_2$ and MeOH extracts. Radioactivity was not found bound to soil particles after the extractions, and total radioactivity extracted from the soil amounted to 98.6 %. That result showed that ^{14}C-MC-LR remained available in the soil 1 h after treatment. Chen et al. (2006a) also observed similar yields with extracting solution of EDTA.

In columns submitted to one single treatment with ^{14}C-MC-LR, the mass-balance analysis of labelled carbon gave similar trends after 28 or 56 days of incubation. Labelled carbon was mainly partitioned among EDTA (31.9 and 50.9 %) and $CaCl_2$ (42.87 and 26.4 %) extracts after each of the two periods. Radioactivity bound to the soil fractions represented only 13–14 % of initial radioactivity, whereas mineralized carbon was 11.5–13.8 %. In the column submitted to two successive treatments with ^{14}C-MC-LR, the analysis gave comparable results. Total radioactivity extracted from the soils amounted to 101.0–104.9 % of initial amounts.

In the light of these results, the ^{14}C-MC-LR or its transformation products appear weakly adsorbed on the soil. Their availability was high in all the columns, although with a small decrease with time according to values measured in the $CaCl_2$ extracts referred as the most available/mobile fractions in the soil. As a consequence, the incubation time seems to decrease the possible lixiviation of toxins during raining events. Our study confirms results previously described in the literature by Eynard et al. (2000) and Chen et al. (2006a). They explained a limited fixation of ^{14}C-MC-LR onto the soil due to its characteristics. Chen et al. (2006b) had already observed low sorption capacity for sandy soil, mainly explained by a small proportion of clay. Our soil was mainly composed of sand, so its sorption capacity was low. In addition, the clays (illite/smectite) were negatively charged at our soil pH (5.6) and were coated with organic matter present in high amounts, exhibiting a negative density of charge. Furthermore, it was also known that charges of MC-LR are negative in a range of pH comprised between 2.19 and 12.48 (de Maagd et al. 1999). As a consequence, the electrostatic interactions between these three components make our toxin very weakly sorbed onto soil particles. That interpretation agrees with a previous study published by Miller et al. (2001).

Analysis of aqueous soil extracts

The radioactivity contained in the aqueous soil extracts ($CaCl_2$, EDTA+$Na_4P_2O_7$ solutions) was totally retained on the MCH-10 column during the online concentration step. Consecutive high-pressure liquid chromatography analysis showed that 58–81 % of the initial radioactivity in the $CaCl_2$ extracts was eluted from the analytical column. That amount approximated only 50 % for EDTA extracts. During all analyses, only two fractions exhibited relevant radioactivity after liquid scintillation counting. They corresponded to a polar compounds eluted after 5 min of analysis (4–5 % of eluted radioactivity) and to ^{14}C-MC-LR (76–82 % of eluted radioactivity).

Transfer of radiolabelled microcystin-leucine-arginine (^{14}C-MC-LR) from soil to tomato seedlings

Tomato seedlings transferred in the soils submitted to two successive treatments with ^{14}C-MC-LR quickly died. By contrast, seedlings survived in the soil submitted to one single treatment during 28 or 56 days, but remaining unthrifty. These results suggest a toxic effect of the MC-LR at the relatively high concentration (18 mg kg^{-1} dry soil) used in our experiments. Nevertheless, seedlings were harvested, pooled and weighted, and the radioactivity determined by combustion. It corresponded to 6 μg MC-LR g^{-1} fresh seedlings, in a similar order of magnitude (1–2 μg g^{-1}) than values recently published in tomato or farm products (Mohamed and Al Shehri 2009; Gutiérrez-Praena et al. 2014).

Conclusion

From the present study based on the use of radiolabelled microcystin-leucine-arginine, it is observed that this heptapeptide cyanotoxin introduced in a silty sand soil undergoes a weak microbial mineralization under aerobic conditions and remains quantified in high amounts in aqueous extracts from the soil. Furthermore, the low mineralization did not result in the alteration of microbial activities, but further studies are required to verify the biodegradation of MC-LR brought during irrigation more realistic at concentrations. If our results allow us to conclude that microcystins may accumulate in the sandy soil after successive inputs in high amounts and may remain mobile within the soil column, these results may differ according to other experimental conditions.

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