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Effects of carbon amendment on *in situ* atrazine degradation and total microbial biomass

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This study elucidates the effects of carbon amendment on metabolic degradation of atrazine (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine) and total microbial biomass in soil. Degradation of ^{14}C-ring-labelled atrazine was monitored in laboratory incubations of soils supplemented with 0, 10, 100 and 1000 μg g^{-1} sucrose concentrations. An experiment to determine the effect of carbon amendment on total microbial biomass and soil respiration was carried out with different concentrations of sucrose and non-labelled atrazine. The soils were incubated at a constant temperature and constant soil moisture at water potential of −15 kPa and a soil density of 1.3 g cm^{-3}. Mineralization of ^{14}C-ring-labelled atrazine was monitored continuously over a period of 59 d in the first experiment. The CO_2 production was monitored for 62 d in the second experiment and microbial biomass determined at the end of the incubation period. The addition of 1000 μg g^{-1} sucrose reduced atrazine mineralization to 43.5% compared to 51.7% of the applied amount for the treatment without sucrose. The addition of 1000 μg g^{-1} sucrose modified the transformation products to 1.08 μg g^{-1} deisopropylatrazine (DIA), 0.32 μg g^{-1} desethylatrazine (DEA) and 0.18 μg g^{-1} deisopropyl-2-hydroxyatrazine (OH-DIA). Treatment without sucrose resulted in formation of 0.64 μg g^{-1} hydroxyatrazine (HA), 0.28 μg g^{-1} DIA and 0.20 μg g^{-1} OH-DIA. Atrazine dealkylation was enhanced in treatments with 100 and 1000 μg g^{-1} of sucrose added. HA metabolite was formed in the control (no sucrose) and in the presence of 10 μg g^{-1} of sucrose, whereas DEA was only detected in treatment with 1000 μg g^{-1} sucrose. Results indicate that total microbial biomass increased significantly ($P < 0.001$) with the addition of 1000 μg g^{-1} sucrose.

Keywords: Atrazine, carbon amendment, mineralization, metabolites, microbial biomass.

Introduction

Atrazine, (6-chloro-N^2-ethyl-N^4-isopropyl-1,3,5-triazine-2,4-diamine), is an s-triazine-ring herbicide that is used globally to control pre- and post-emergence broad-leaf and grassy weeds in major crops. The prevalence of atrazine-contaminated water and the increased concern about the herbicide has led to many studies on bioremediation of atrazine-contaminated soils.[1] A pesticide may initially persist longer when it is first introduced to the soil.[2] For instance, atrazine was initially considered recalcitrant.[3] However, studies from the 1990s onwards reported adaptation of agricultural soils to atrazine degradation, which later resulted in the isolation of microbes that were capable of degrading the herbicide.[3] In subsequent years, acclimation of the soil micro flora to atrazine mineralization after repeated applications of the herbicide under field conditions was observed.[1,4–9] Subsequently, there have been several reports of complete and rapid atrazine mineralization in agricultural soils,[6,7,10] and a variety of atrazine-mineralizing bacteria, including members of the genera *Pseudomonas*, *Agrobacterium*, and *Arthrobacter* have been isolated from geographically diverse locations in soils that have come in contact with the chemical.[8,11–16] Microorganisms can use the pesticide as a carbon source, or, in the case of co-metabolism, another energy source is used which may or may not be structurally related to the pesticide molecule. All microbiological reactions are catalyzed by enzymes which are either normally present in the organisms or are induced by the pesticide, and may also be present as free exo-enzymes in the soil.[17] The use of the ring-substituent C has been reported and microbial growth measured with atrazine as the sole C source.[12,18] Evidence

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of rapid triazine ring mineralization, however, implies the development of microbial communities that can utilize the N of the triazine ring as an N source.[13,14,19]

Atrazine mineralization is achieved in two stages. Initially, the chlorine and *N*-alkyl side-chains are removed to yield cyanuric acid (2,4,6-trihydroxy-1,3,5- triazine). The enzymes involved in this stage often have broad substrate specificity and may perform a variety of reactions on different substrates.[2] The *s*-triazine ring is then cleaved and subsequently converted to ammonia and carbon dioxide by a set of enzymes that have a much narrower substrate range. However, ring cleavage apparently occurs only after hydroxylation.[20] Conversion of atrazine to cyanuric acid can be achieved via three distinct pathways, one of which is purely hydrolytic, while the other two are mixed oxidative-hydrolytic. The hydrolytic pathway has been extensively characterized in *Pseudomonas* sp. ADP, and consists of three enzymatic steps catalyzed by the gene products of *atzA*, *atzB* and *atzC*.[21] Oxidative-hydrolytic pathways involve the initial oxidative N-dealkylation of atrazine to deisopropylatrazine or deethylatrazine. The initial dealkylated products may be further dealkylated to deisopropyldeethylatrazine, or may be subjected to hydrolytic dechlorination, deamination and/or dealkylation to yield cyanuric acid. Cyanuric acid is a common intermediate of many *s*-triazine biodegradation pathways[22] and its metabolization invariably occurs via hydrolytic cleavage of the *s*-triazine ring and consecutive hydrolysis of biuret and allophanate to produce ammonia and carbon dioxide.[23]

Biostimulation has been adopted as an effective bioremediation strategy that involves modification of the environment to stimulate existing microbes capable of degrading the pesticide.[24,25] This has been achieved by the addition of various forms of limiting nutrients, such as carbon, phosphorus, or nitrogen. Comprehensively, biostimulation could be perceived as the introduction of adequate amounts of water, nutrients, and oxygen into the soil, in order to enhance the activity of indigenous microbial degraders or to promote co-metabolism.[26]

One of the prominent biostimulation methods is the addition of organic amendments.[27] The addition of organic amendments frequently modifies the rates and pathways of pesticide degradation in soils according to the nature and the reactivity of the organic amendments and their effect on microbial activity.[28,29] Co-metabolic biotransformation can be enhanced by the general increase in microbial activity stimulated by the organic matter.[30] For atrazine, organic amendments added to soil often decreased atrazine mineralization due to increased sorption of the herbicide.[31] Mostly, nutrients in the soil stay below optimal concentration for microbial activity. Supplementing such soils with the necessary nutrients instigates the biodegradation of the pollutants and is a promising technique to enhance the bioremediation of contaminated sites. Nutrients like carbon, nitrogen, and phosphorus stimulate microbes to create the essential enzymes to break down the contaminants.[32] In some cases, inorganic nitrogen starvation may be more effective in promoting degradation. Such an approach has been reported for atrazine and other heterocyclic compounds. This can potentially be accomplished by supplying excess carbon to make nitrogen limiting.[33,34]

The aim of this study was to evaluate the effects of increased carbon amendment from sucrose on metabolic degradation of atrazine. The use of sucrose was appropriate as the soils used in the study were from a sugarcane-cultivated field. The effects of increased microbial biomass on atrazine degradation were also assessed.

Materials and methods

Soils

The soil material was collected from a sugarcane-cultivated field in Nzoia (34° 34′ 00″ to 34° 51′ 30″ E and 0° 23′ 00″ to 0° 37′ 30″ N), Kenya. The relevant soil characteristics were: clay 13%, silt 15%, sand 72%, organic C 0.80%, total N 0.05%, pH ($CaCl_2$) 5.1, CEC 142 mmol kg^{-1}, P 8.23 mg $kg^{-1}$and S 78.33 mg kg^{-1}. Before the experiments were started, the soil samples (depth 0–20 cm) were sieved (<2 mm) and kept at room temperature (20 ± 1°C) for 5 d after moistening to a water potential close to but below −15 kPa.

Chemicals

The chemical ^{14}C-ring-labelled atrazine, with a specific radioactivity of 351.5 MBq $mmol^{-1}$ and a radiochemical purity of >98% was purchased from Sigma–Aldrich (St. Louis, MO, USA). Non-labelled atrazine and the metabolite standards deethylatrazine (DEA), deisopropylatrazine (DIA), deethyl-deisopropylatrazine (DEDIA), hydroxyatrazine (HA), deethyl-2-hydroxyatrazine (OH-DEA), deisopropyl-2-hydroxyatrazine (OH-DIA), and deethyldeisopropyl-2-hydroxyatrazine (OH-DEDIA) were obtained from Ehrenstorfer (Augsburg, Germany). All chemicals had a purity of 99%. Scintillation cocktails were obtained from Packard (Dreieich, Germany). All other chemicals and solvents were of analytical grade and were purchased from Merck (Darmstadt, Germany).

Degradation experiments

Pesticide and sucrose application. Degradation experiments on ^{14}C-ring- labelled atrazine were carried out in an aerated closed laboratory system as described previously by Schroll and Kühn[35] with a 10 g soil (dry weight) in 100 mL double walled glass incubation vessels. Non-labelled and ^{14}C-labelled atrazine were mixed and dissolved in methanol to give a final specific radioactivity of 66.3 Bq μg^{-1}. A volume of 31 μL of this application standard with a radioactivity of 8.46 kBq was applied dropwise with a

Hamilton syringe to an aliquot of 1.0 g of oven-dry, ground soil. Aliquots of 0.1 mL of prepared sucrose stock solutions were applied to the same aliquot of 1.0 g oven-dry, ground soil giving concentrations of 1000, 100 and 10 μg sucrose/g of soil. The aliquot was stirred with a spatula for 2 min until a homogenous distribution of the herbicide was achieved. After evaporation of methanol the aliquot was mixed for another period of 2 min with fresh dry soil (9.0 g) yielding a total sample amount of 10 g dry soil per experiment with a pesticide concentration of 25 mg kg^{-1}. This concentration corresponds to an agronomic application rate of 1250 g ha^{-1} when assuming that the distribution of the herbicide might be at 3–4 mm depth shortly after the application in a field with a soil density of approximately 1.3 g cm^{-3}. The experiments were conducted in four replicates with a parallel set without sucrose treatment. The soils were then transferred to the incubation flasks, compacted to a density of 1.3 g cm^{-3} and water adjustment done to a water potential of -15 kPa (pF 2.13) to obtain maximum pesticide mineralization in the soils.[36]

The mineralization of the ^{14}C-ring labelled atrazine was monitored by quantifying the $^{14}CO_2$ released. At regular time intervals (three times a week) the incubation flasks were connected to the closed biodegradation systems filled with 10 mL 0.1 M NaOH to trap the accumulated $^{14}CO_2$ released to the headspace by flushing sterile $^{14}CO_2$-free air through the trapping system. A 2 mL aliquot of the absorption liquid, 1.0 M NaOH, was withdrawn and mixed with 3 mL of scintillation cocktail Ultima Flo AF (Packard, Dreieich, Germany). Radioactivity was detected by scintillation counting.

After 59 days of incubation of the soils, aliquots of soil samples were extracted by ASE method and extracts cleaned up by SPE. The extracts obtained after SPE clean up procedure were analyzed for atrazine and metabolites residues. Soil samples that had been subjected to extractions were analyzed for non-extractable residues.

Solvent extraction of soil, clean up and analysis

At the end of the incubation period, the soil samples were extracted with methanol in an accelerated solvent extractor (ASE 200, Dionex, Idstein, Germany) at 90°C, with a pressure of 10 MPa.[37] Aliquots of 0.5 mL of each extract were mixed with 4.5 mL Ultima Gold XR and measured by liquid scintillation counting. Subsequently, extracts were concentrated with a rotary evaporator to a volume of 2-3 mL. Efficiency of the ASE extraction procedure was evaluated by spiking two 10 g soil (dry weight) samples with 31 μL (25 mg kg^{-1}) of the application standard with a radioactivity of 8.46 kBq. Based on radioactivity, the recoveries ranged between 89–94%.

The concentrated methanolic soil extracts were dissolved in 250 mL distilled water. The methanolic extracts were cleaned up with Isolate Triazine columns (500 mg, Separtis, Grenzach-Wyhlen, Germany). After extraction, the SPE columns were dried under a gentle nitrogen-stream and eluted with 10 mL methanol. The eluate was concentrated to a volume of 1 mL with a rotary evaporator and further concentrated to a volume of 0.2 mL under a gentle nitrogen-stream. The samples were immediately analyzed by HPLC or stored at −20°C before analysis. Efficiency of SPE procedure was evaluated by measuring the radioactivity of extract samples before and after the clean-up procedure. The recoveries based on radioactivity measurement ranged from 91–96%.

For residue analysis 20 μL of each soil extract was injected to a HPLC system that was equipped with a L-6200 Intelligent Pump (Merck-Hitachi, Darmstadt, Germany), a UV/VIS detector (220 nm, Merck-Hitachi, Darmstadt, Germany), and a radioactivity detector LB 506 C1 (Berthold, Wildbad, Germany); column: LiChrospher 100 RP-18, 2 tm, 4 × 250 mm (Merck- Hitachi, Darmstadt, Germany). The mobile phase consisted of 0.003 M KH_2PO_4, pH 3 (A) and acetonitrile (B) at a flow rate of 0.8 mL min^{-1}. The gradient program was: T0 min 20% (A); T10 min 38% (A); T24 min 75% (A); T29 min 75% (A); T33 min 20% (A); T40 min 20% (A). Parent compound and metabolites were identified by comparison of their retention times with reference standards. Under these conditions, atrazine and its metabolites eluted at R_t values 14.9, 8.5, 6.1, 3.9, 4.6, 5.9, 2.5 and 3.3 minutes for atrazine, DEA, DIA, OH-DIA, OH-DEA, HA, DEIA and OH-DEIA, respectively. The detection limits of the method (based on radioactivity detection) were as follows: atrazine: 23.5 μg kg^{-1} soil (dry weight); DEA: 20.5 μg kg^{-1}; DIA: 18.9 μg kg^{-1}; OH-DIA: 16.9 μg kg^{-1}; OH-DEA: 18.6 μg kg^{-1}; HA: 17.4 μg kg^{-1}; DEIA: 19.7 μg kg^{-1} OH-DEIA: 17.5 μg kg^{-1}.

Quantification of non-extractable residues

After ASE, the soil material was dried and homogenized intensively. Three aliquots of each soil sample were filled into combustion cups and mixed with 3-4 drops of saturated aqueous sugar solution to guarantee a complete oxidation of the ^{14}C. The combustion was conducted with an automatic sample-oxidizer 306 (Packard, Dreieich, Germany). The compound $^{14}CO_2$ was trapped in Carbo- Sorb E (Packard, Dreieich, Germany) and mixed with Permafluor E (Packard, Dreieich, Germany) prior to scintillation counting. The detection limit for non-extractable residue (NER) was 9.0 μg kg^{-1} dry soils.

Determination of effect of increased carbon amendment on soil respiration

Parallel to the soil incubation experiment with different sucrose concentrations, a similar soil incubation experiment was conducted and the total CO_2 evolved from non-labelled atrazine was measured during an incubation period of 62 days.

The experiment was carried out with a 20 g dry soil sample. Atrazine standard, 0.05μL, from a stock solution (100 mg/10 mL) was applied to 1.0 g of oven-dry, ground soil in a 50 mL beaker using a Hamilton syringe and mixed thoroughly for 2 minutes to ensure a homogenous mixture. From a stock solution of 2×10^5 mg mL^{-1} sucrose, appropriate aliquots were added to the 1 g soil samples corresponding to 1000, 100 and 10 μg g^{-1} sucrose concentrations, respectively. The soil aliquots were mixed thoroughly for another period of 2 min with fresh soil (19.0 g dry weight) yielding a total sample amount of 20 g dry soil per experiment with 25 μg g^{-1} atrazine concentration. A total of eight treatments were prepared as shown in Table 1. The 20 g soil aliquots were compacted in 50 mL beakers to give a soil density of 1.3 g cm^{-1} and water content was adjusted to a water potential of -15 kPa (pF 2.13).

The compacted soils in the 50 mL beakers were incubated in a respirometer (Respicond IV, Nordgren, Innovations, Sweden). The evolved CO_2 from respiration was trapped by 0.6 M KOH solution in the reaction cells. The reaction between the KOH and the evolved CO_2 as a result of the basal respiration of the indigenous soil microbes resulted in changes in conductivity which was monitored and measured after every 30 minutes. The measurements were conducted in a water bath maintained at 20°C. A plot of the total CO_2 (in mg) measured against time was obtained. The rate of CO_2 formation (CO_2 mg d^{-1}) was computed from the amount collected during the incubation period. A second addition of the sucrose was done for the treatments after 30 days of incubation. At the end of the experiment, microbial biomass in the soil at different sucrose concentrations was determined by vacuum chloroform fumigation extraction method.[38]

Determination of microbial biomass in the treated soil

All the eight treatments described in the previous section (Table 1) were used for soil microbial biomass measurements. Soil microbial carbon was measured by a slight modification of the original method proposed by Jenkinson and Powlon.[38] Aliquots of 4 g equivalent of dry soil were weighed into 20 mL extraction glass vials. Two sets of soil samples were prepared, one set for fumigation and the other as a control with four replicates for each of the eight treatments.

Table 1. Description of the treatments with different sucrose concentrations.

Treatment no.	*Treatment description: 20 g soil, dry weight, with:*
T_1	no glucose or atrazine
T_2	25 μg g^{-1} atrazine
T_3	10 μg g^{-1} sucrose and 25 μg g^{-1} atrazine
T_4	10 μg g^{-1} sucrose
T_5	100 μg g^{-1} sucrose and 25 μg g^{-1} atrazine
T_6	100 μg g^{-1} sucrose
T_7	1000 μg g^{-1} sucrose and 25 μg g^{-1} atrazine
T_8	1000 μg g^{-1} sucrose

The non-fumigated (control) set of soil samples was immediately extracted with 16 mL 0.5 M K_2SO_4 solution by shaking for 45 minutes in an oscillating shaker. After shaking, the mixture was filtered using pleated Whatman No. 42 filters that had been pre-washed twice with milli Q water. The filtrate was frozen at -18°C until the total organic carbon (TOC) was determined. The other set of soil samples was fumigated as follows: the soil samples in four replicates were placed in a desiccator lined with moist paper towels at the bottom. A 25 mL of methanol-free chloroform was placed in a flask with boiling chips. Another flask with soda lime pellets was placed on the moist paper towel. The soil samples were placed in the vacuum desiccator, which was sealed in a laboratory hood and evacuated with a vacuum pump, allowing the chloroform to boil for 2 minutes. The desiccator was then saturated with chloroform vapour and left with the soil samples for 24 hours.

At the end of the chloroform- incubation period, the desiccator was air-flushed several times to remove the chloroform. The glass vials with the fumigated samples were removed from the desiccator. An aliquot of 1 mL of 0.5 M K_2SO_4 solution was added to each fumigated soil sample in the glass vial and shaken for 45 minutes. The mixture was filtered and frozen at -18°C until TOC was determined. TOC was analyzed by the TOC Analyzer (Dimatec 100 Analysentechnik, Germany). A calibration curve for total carbon was prepared using potassium hydrogen phthalate.

To check on the reliability of the TOC measurement data, the total inorganic carbon (TIC) and total carbon (TC) for a mixture of sodium carbonate and sodium hydrogen carbonate at concentrations of 15 and 5 mg L^{-1}, respectively, was obtained. The mixture was included in the sample measurements. An aliquot of 1 mL of the 0.5M K_2SO_4 solution extracts of the fumigated and non-fumigated soils was diluted four and two times, respectively, in plastic vials. The samples were measured together with the TIC and TC standards.

From the measurements, the carbon released by microbes during chloroform fumigation was computed and the microbial biomass was determined using the following equation: C (biomass) = 2.64*E_C, where C is the carbon associated with the biomass cells, 2.64 is K_C factor commonly used for aerobic soils as described by Valence et al.[39] and E_C is the difference between C extracted from the fumigated and non-fumigated treatments.

Results and discussion

Effect of carbon amendment on ^{14}C Atrazine mineralization

The soils used for this study had microbes with a high capacity for mineralizing atrazine as indicated from a liquid

culture experiment that showed 72% (of applied amount) mineralization of ^{14}C-ring-labelled atrazine after 76 d incubation period. Application of different amounts of sucrose resulted in differences in % mineralization of atrazine (Fig. 1). Soils with no sucrose treatment showed 51.7% atrazine (% of applied amount) mineralization after 59 days of incubation. The addition of 10 μg g^{-1} sucrose had no effect on % mineralization as the there was no significant difference ($P = 0.73$) between this variant and control (no sucrose). However, addition of 100 μg g^{-1} and 1000 μg g^{-1} resulted in reduced mineralization of ^{14}C-atrazine of 47.2% and 43.5% of applied amount, respectively after 59 days incubation.

This mineralization behaviour suggests that the addition of mineral carbon to an adapted soil did not enhance ^{14}C-atrazine mineralization. Instead, the addition of 1000 μg g^{-1} sucrose lowered significantly ($P < 0.0001$) the mineralization. This may be due to increased sorption of atrazine by the increased biomass and soil matrix.[31] Studies have been carried out on the effect of increasing availabilities of carbon and nitrogen on atrazine behaviour in soils. Such studies have reported both positive and negative effects on atrazine mineralization as a result of organic and inorganic amendments in soils. A study by Assaf and Turco[40] observed that addition of mannitol (carbon source) to the soil after 140 days increased the $^{14}CO_2$ evolution as a result of enhanced mineralization of atrazine. Atrazine mineralization was enhanced after soil had been amended with 1000 mg kg^{-1} of mannitol in a study by Stucki et al.[41] Likewise, there have been reports of enhanced mineralization of atrazine following the addition of glucose.[42] The effects of added carbon on atrazine degradation may be the indirect result of making nitrogen limiting, thereby enhancing access of atrazine as a nitrogen source.[26]

On the other hand literature mentions the possible inhibitory effects of organic amendments and nutrient addition on herbicide mineralization in soil. Ghosh and Philip[43] observed the effects of external carbon source on atrazine degradation by mixed culture anaerobic bacteria. In co-metabolic process with dextrose as the primary source of carbon and energy, there was a certain ratio of primary substrate and the toxic compound, at which degradation of atrazine was at maximum. When dextrose was about 2000 mg L^{-1} , atrazine degradation reduced drastically, which might have been due to the inhibition on the secretion of atrazine degrading inducible enzyme. In studies by Abdelhafid et al.[4] with an adapted soil and a non-adapted soil, treatment with the largest concentration of added glucose decreased significantly the mineralization of atrazine. In another study, insufficient total assimilable organic carbon and or inorganic nutrients had a negative effect on s-triazine degradation by co-substrate or co-metabolic processes.[44] Similarly, in this study, addition of 1000 μg g^{-1} decreased atrazine mineralization. Organic and inorganic amendments favoring the sorption of herbicides can reduce their microbial bioavailability and repress their biodegradation.[29,31] Therefore, increased sorption of atrazine by the increased soil biomass and soil matrix resulted in decreased mineralization. Also, the competition for space and nutrients between atrazine-degrading microorganisms and the total heterotrophic micro flora may contribute to the decrease in atrazine mineralization as proposed in studies by Abdelhafid et al.[4] The decrease in atrazine mineralization by 1000 μg g^{-1} sucrose is corroborated by the high amount of non-extractable atrazine as shown below.

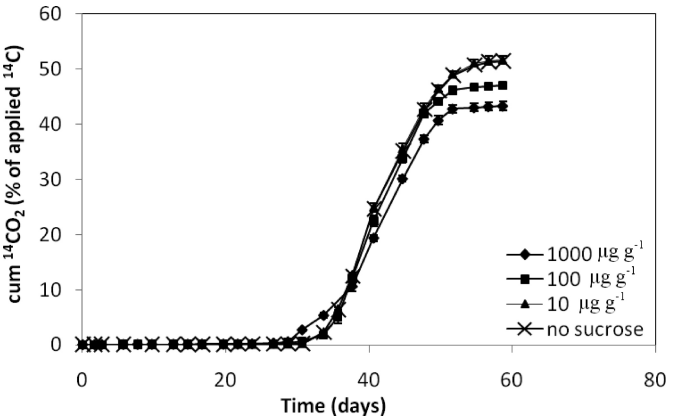

Fig. 1. Effect of increasing sucrose addition on mineralization of ^{14}C-atrazine in soil.

Effects of increasing carbon on extractable and non-extractable residues

The largest concentration of sucrose (1000 μg g^{-1}) decreased significantly ($P < 0.0001$) the mineralization of atrazine compared to the control (no sucrose). Simultaneously, the non-extractable residues increased significantly. There was no significant difference ($P = 0.12$) in amounts of extractable residues between the control and the 10 μg g^{-1} sucrose variant. Compared to the other treatments, the addition of 1000 μg g^{-1} sucrose resulted in a reduced amount of extractable residues of 4.3 μg g^{-1}. There was a significant difference between the extractable residues from the control and those in variants with 100 μg g^{-1} ($P = 0.02$) and 1000 μg g^{-1} ($P = 0.002$) sucrose, respectively. Consequently, the amounts of non-extractable residues formed in 1000 μg g^{-1} variant were significantly ($P < 0.0001$) higher than control. Also, the variant with 100 μg g^{-1} sucrose resulted in formation of non-extractable residues that were significantly different ($P = 0.001$) from the control. However, there was no significant difference ($P = 0.48$) in non-extractable residues formed by the control and variant with 10 μg g^{-1} sucrose (Table 2). There was a good agreement on % recoveries from the mass balances and the applied amount of atrazine.

The increased formation of non-extractable residues for the treatment with the highest sucrose concentration of 1000 μg g^{-1} may be due to the retention of atrazine by

Table 2. Mass balance for atrazine at different sucrose concentrations.

Parameter	*Different sucrose concentrations*			
	no sucrose	*10 μg g^{-1} sucrose*	*100 μg g^{-1} sucrose*	*1000 μg g^{-1} sucrose*
Mineralization (μg g^{-1})	13.1 ± 0.3	13.1 ± 0.4	11.9 ± 0.1	11.0 ± 0.6
Extractable residues (μg g^{-1})	5.0 ± 0.7	5.2 ± 0.5	5.1 ± 0.6	4.3 ± 0.3
Non-extractable residues (μg g^{-1})	6.3 ± 0.4	6.4 ± 0.3	7.3 ± 0.1	9.6 ± 0.2
Total %	97.5 ± 0.8	99.2 ± 2.5	97.6 ± 1.5	99.7 ± 2.4

n = 4 ± standard deviation

the growing microbial biomass after sucrose addition. In a study by Houot et al.,[31] the addition of municipal compost increased atrazine sorption and decreased its availability for degradation by soil microorganisms. In that study, at the end of the incubation, less atrazine was mineralized in the presence of the two types of organic amendments, municipal compost and composted straw, which both increased the formation of non-extractable residues of atrazine. Similarly, the increased microbial biomass in the variant with 1000 μg g^{-1} observed in this study resulted in an increase in atrazine retention. This in turn resulted in an increase in formation of non-extractable residues for this treatment.

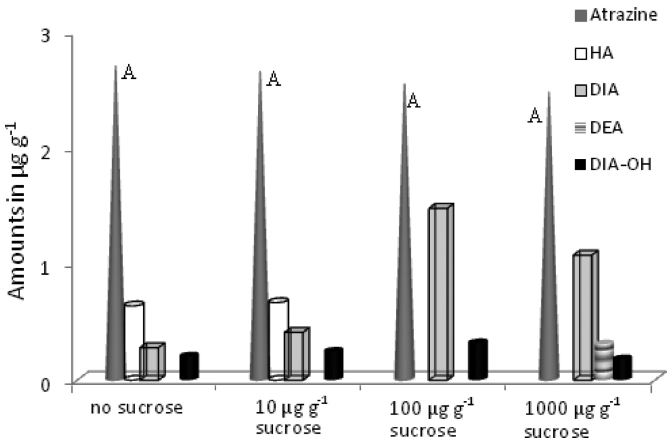

Fig. 2. Distribution of ^{14}C of atrazine and metabolites in extractable residues. A represents atrazine (color figure available online).

Modification of metabolites formation from atrazine by sucrose concentration

It was observed that the addition of different amounts of sucrose resulted in modification of the metabolites formed. Atrazine was the dominant compound in the extractable fraction and mainly dealkylated metabolites were detected with very little hydroxyatrazine. The variant with the addition of 10 μg g^{-1} sucrose resulted in formation of HA, DIA and OH-DIA metabolites similar to that of the control. However, the amounts of DIA formed by the variant with 10 μg g^{-1} sucrose added were slightly higher (0.41 μg g^{-1}), than control (0.28 μg g^{-1}). HA metabolite was formed only in the control (no sucrose) and the variant with 10 μg g^{-1} sucrose (Fig. 2). Therefore, addition of 10 μg g^{-1} and treatment with no sucrose favoured initial hydroxylation with less dealkylation of atrazine

The addition of 100 μg g^{-1} sucrose modified the concentration of the metabolites resulting in formation of DIA

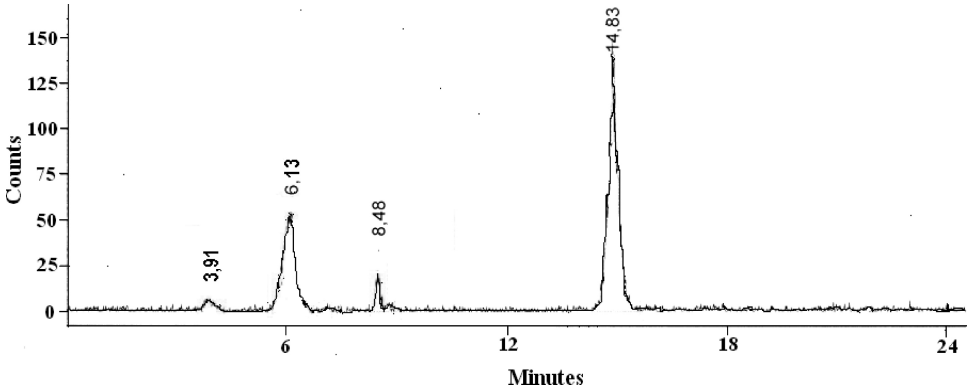

Fig. 3. HPLC chromatogram for extractable atrazine and metabolite residues in soils incubated with 1000 μg g^{-1} sucrose and 25 μg g^{-1} atrazine. The peaks represent: Rt 14.58 is atrazine with 2.46 μg g^{-1}; Rt 8.48 is DEA with 0.31 μg g^{-1}; Rt 6.13 is DIA with 1.08 μg g^{-1} and Rt 3.91 is OH-DIA with 0.18 μg g^{-1}.

(1.48 μg g^{-1}) and OH-DIA (0.31 μg g^{-1}). Similarly, the variant with the addition of 1000 μg g^{-1} sucrose modified the metabolites formation in the soils. This treatment resulted in formation of DIA (1.08 μg g^{-1}), OH-DIA (0.18 μg g^{-1}) and DEA (0.32 μg g^{-1}) metabolites (Fig. 2). Figure 3 is a sample chromatogram for extractable atrazine and metabolite residues for the variant with 1000 μg g^{-1} sucrose addition, showing formation of DIA, OH-DIA and DEA.

The results suggest that the sucrose amendment may have an effect on the enzymatic activities. Similar studies by Houot et al.[31] on modifications to atrazine degradation pathways in loamy soils after the addition of organic amendments established that both organic amendments (a municipal solid waste compost and a composted straw), modified the behavior of atrazine in soil but via different processes. In this study, less sucrose and no addition of sucrose favoured formation of HA. The hydroxylation favoured the opening of the triazine ring and its subsequent mineralization in the soil through hydrolytic pathway.[45] Lesser amounts of atrazine were degraded through the oxidative-hydrolytic pathway. On the other hand, increased amount of sucrose favored the increased formation of DEA and DIA and subsequent formation of DIA-OH through oxidative-hydrolytic pathways. Dechlorination of DIA to the respective hydroxylated compound OH-DIA was demonstrated by Behki and Khan.[18] OH-DIA is an important intermediate product in atrazine mineralization since ring cleavage apparently occurs only after hydroxylation.[20] The findings from this study suggest that low sucrose concentration in adapted soil favours atrazine mineralization whereas increased sucrose inhibits mineralization and modify the metabolites formation.

Effect of increased C amendment on soil respiration and soil microbial biomass

Incubation of the soils with different sucrose concentrations and 25 μg g^{-1} atrazine for 62 days showed an increased production of CO_2 for the variant with1000 μg g^{-1} sucrose addition (Fig. 4a and 4b). There was no significant difference ($P > 0.05$) in CO_2 production for treatments that had the same amount of sucrose but with or without 25 μg g^{-1} atrazine. The cumulative CO_2 in mg for treatments with 1000 μg g^{-1} (150.6 mg) sucrose was four times higher than that for 100 μg g^{-1} (40.4 mg) and 10 times higher for the 10 μg g^{-1} (14.9 mg) sucrose treatments after the 62 days incubation period. Therefore, the addition of sucrose increased the soil respiration with the effect of 25 μg g^{-1} atrazine addition being undetectable.

The rates of CO_2 production (CO_2 mg d^{-1}) were highest for the variant with 1000 μg g^{-1} sucrose addition achieving a rate of 4.3 mg d^{-1} that dropped to an average rate of 3.3 mg d^{-1} after 10 days of incubation. This increase was

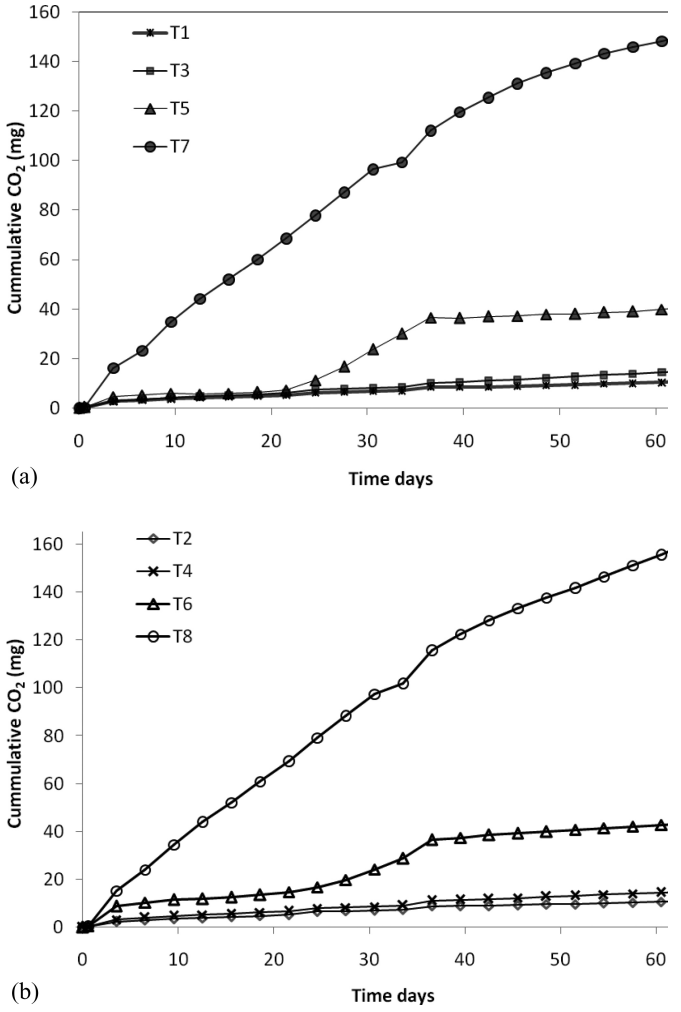

Fig. 4. CO_2 production at different sucrose concentrations. T_1, T_3, T_5 and T_7 are the different treatments of 20 g soils, dry weight: T_1 is soils alone; T_3 is soil and 10 μg g^{-1} sucrose; T_5 is soil and 100 μg g^{-1} sucrose; T_7 soil with 1000 μg g^{-1} sucrose. (b). CO_2 production at different sucrose concentrations. T_2, T_4, T_6 and T_8 are the different treatments of 20 g dry soils: T_2 is soil and 25 μg g^{-1} atrazine; T_4 is soil, 10 μg g^{-1} sucrose and 25 ppm atrazine; T_6 is soil, 100 μg g^{-1} sucrose and 25 μg g^{-1} atrazine; T_8 is soil, 1000 μg g^{-1} sucrose and 25 μg g^{-1} atrazine.

Table 3. Rate of CO_2 production for treatments with different sucrose and atrazine concentrations at 4 days incubation period.

	Rate of CO_2 production (mg d^{-1})	
Sucrose conc. (μg g^{-1})	*0 μg g^{-1} atrazine*	*25 μg g^{-1} atrazine*
0	0.75 ± 0.06	0.69 ± 0.11
10	0.79 ± 0.34	0.92 ± 0.07
100	2.27 ± 0.13	2.49 ± 0.22
1000	4.53 ± 0.21	4.30 ± 0.40

n = 4 ± standard deviation

Table 4. Microbial biomass measurements for treatments with different sucrose and atrazine concentrations.

	Microbial biomass (C biomass)	
Sucrose conc. ($\mu g\ g^{-1}$)	*0 $\mu g\ g^{-1}$ atrazine*	*25 $\mu g\ g^{-1}$ atrazine*
0	0.76 ± 1.20	1.23 ± 0.60
10	2.38 ± 0.43	2.47 ± 0.71
100	12.99 ± 0.52	11.76 ± 1.02
1000	16.26 ± 0.90	18.80 ± 0.46

n = 4 ± standard deviation

due to increased microbial growth. Production of CO_2 was highest within the first three to four days of incubation for all the treatments. The rates of CO_2 (mg d^{-1}) production after 4 days of incubation are given in Table 3.

The soil microbial biomass for the different treatments was determined after 62 days of soil incubation through chloroform fumigation method. The microbial biomass was significantly high ($P < 0.001$) for the treatments with 1000 μg g^{-1} sucrose addition. Increase in sucrose resulted in increased total microbial biomass. Treatments with the same amount of sucrose but with or without atrazine addition showed no significant difference ($P > 0.05$) in microbial biomass measured (Table 4).

The addition of sucrose increased soil respiration, thus increasing total carbon mineralization and total microbial biomass. The effect of atrazine addition on soil respiration and total microbial biomass was not detected. Studies by Abdelhafid et al.[4] with an adapted and non-adapted soil to atrazine mineralization showed that total organic C mineralization increased with the addition of glucose in both soils and that total C mineralization and the microbial biomass were influenced more by glucose addition than by mineral N.

The addition of sucrose stimulated and enhanced the microbial growth resulting in increased total C mineralization and microbial biomass. Therefore, the observed increase in the soil respiration and total microbial biomass is in agreement with earlier findings.[4]

Conclusion

In this study with a soil adapted to atrazine, sucrose amendment at a concentration 1000 μg g^{-1} did not enhance mineralization. This amount of sucrose resulted in increased formation of non-extractable residues. Increased microbial biomass may have resulted in increased sorption of atrazine, thereby decreasing its availability for mineralization. However, it was observed that different amounts of sucrose resulted in modification of the metabolites formed. The amounts of these metabolites also varied. The complex soil dynamics and effects of sucrose on enzymatic activities of the degraders may have contributed to the observed results in this study.

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