Introduction

In recent years, there has been a high demand for increasing agricultural productivity and the arable land area, accompanied by the large scale use and discovery of new pesticides and fertilizers [1–3]. It was estimated that approximately 2.27 million tons of agrochemicals were released into the environment in 2001, 35% of which were herbicides [4]. Despite the fact that the use of pesticides in agriculture has had a positive impact on crop productivity, concerns have been expressed about the adverse effects of these chemicals [5], since only 0.1% of them reach their specific targets. For this reason, there is a large quantity of herbicide residues remaining in the environment, which can be metabolized by microorganisms [6–8].

Herbicide application has brought damage to the soil microbiota, and may have affected the dynamics of biogeochemical cycles and soil fertility. The herbicide napropamide, for example, has been identified as harmful to soil functionality, based on the structural and functional diversity of the soil bacterial community [9]. Other studies have demonstrated the inhibition of nitrification and changes in ammonia oxidation in soils by the herbicide simazine [10]. As regard to the triketone herbicides mesotrione and sulcotrione, their toxicity level was considered equal to or higher than atrazine in studies with model organisms, such as Tetrahymena pyriformis and Vibrio fischeri [11].

Bioremediation has been the main strategy used to eliminate xenobiotics, mainly herbicides, from the environment, and this subject has been the focus of many biotechnological studies [12–13]. Degradation processes mediated by microorganisms in large part influence the persistence of herbicides in the soil [14].

Reactive oxygen species (ROS), apart from being part of normal aerobic metabolism [15], may increase in concentration as a result of exposure to toxic substances, as for example, when bacteria come into contact with herbicides [16]. An increase in the rate of
Mesotrione Tolerance and Degradation by Escherichia coli

Materials and Methods

Bacterial strain

The Escherichia coli strain DH5-α [genotype: F− φ80dlacZAM15 ΔlacZYA-argF U169 deoR, recA1 endA1 hsdR17 (rK− m−) phoA supE44 lac− thi-1 gyrA96 relA1] was used to evaluate the degradation of mesotrione herbicide.

Herbicides

Mesotrione (99% purity) was provided by Syngenta Crop Protection, Greensboro, NC (USA). For HPLC (High Performance Liquid Chromatography) experiments, the analytical standard Pestanal (99% pure) (Sigma-Aldrich) was used.

Evaluation of herbicide degradation by HPLC

In order to determine the mesotrione degrading ability of E. coli, the strain was grown in 100 mL of LB (Luria Broth: 10 g L−1 tryptone; 5 g L−1 yeast extract; 10 g L−1 NaCl) in 250 mL flasks, and incubated at 37°C, at 200 rpm. The cells were collected after 10 h and centrifuged at 8,000 x g for 10 min, at 4°C. The pellet was washed twice with PBS (pH 6.8), phosphate buffered saline: 8 g L−1 NaCl; 0.2 g L−1 KCl; 1.44 g L−1 Na2HPO4; 0.24 g L−1 KH2PO4, and cells were re-suspended in 10 mL of MMM (Mineral Medium with Mesotrione: 3 g L−1 NaNO3; 0.5 g L−1 MgSO4; 0.5 g L−1 KCl; 0.01 g L−1 FeSO4; 0.04 g L−1 CaCl2; 0.001 g L−1 MnSO4; 0.4 g L−1 glucose, 0.04 mM mesotrione; 10 mM potassium phosphate buffer, pH 7.0). This corresponded to 1×, or normal field levels according to manufacturer’s instruction. Cells were placed in mineral medium without carbon source (–CM), with five repetitions, and incubated at 37°C, at 200 rpm. For analysis of herbicide adsorption by bacteria, cells were boiled prior to being added to the treatments already described. As a negative control, a flask with MMM was incubated, in the same conditions, without E. coli DH5-α. Aliquots (1 mL) were collected from the culture medium every hour of incubation (from 0 to 12 h) and centrifuged at 13,000 × g for 5 min. The supernatant (0.9 mL) was frozen for further HPLC analysis.

Samples were filtered with 0.22 μm syringe filters and HPLC analysis was performed using a Waters Alliance e2695 and a photodiode detector (Waters 2998 PDA), adjusted at a wavelength of 254 nm. An Eclipse XDB-C18 column was used, with a flow rate of 1 mL min−1. The injection volume of the samples was 50 μL. The HPLC method was developed, validated and applied to analysis of mesotrione degradation rate.

Cell viability

Bacteria were grown for 10 h at 37°C in 1.2 L of LB. Cells were centrifuged at 8,000 x g for 10 min., washed twice with PBS, and divided into vials containing 50 mL of MM (control), MMM (mineral medium with mesotrione), -C (MM without carbon source) and -CM (MMM with mesotrione as sole carbon source), with three repetitions. The flasks were incubated at 37°C (at 200 rpm), and 100 mL aliquots were withdrawn after 30 min, 3 h and 6 h of incubation. Samples were diluted to 10−6, plated on LB medium and incubated at 37°C. After 12 h, the colony forming units (CFU) counting was determined.
Lipid peroxidation

Lipid peroxidation was determined by the levels of malondialdehyde (MDA) (substances reactive to thiobarbituric acid) [37]. The concentrations of MDA were monitored at 535 and 600 nm, and their concentrations were calculated using an extinction coefficient of 155 mM cm⁻¹.

Protein extraction for analysis of oxidative stress

Bacteria were grown for 10 h at 37°C (at 200 rpm) in 3.6 L of LB, to get enough cells for the following steps of protein extraction. The cells were centrifuged at 8,000×g for 10 min, washed twice with PBS, and divided into vials with 30 mL of MM (control), MMM, -C (MM without carbon source), and -CM (MMM with mesotrione as sole carbon source), with three replications. All the flasks were incubated at 37°C, and extractions were performed at 30 min., 3 h, and 6 h.

For enzyme extraction, the cultures were centrifuged at 8,000×g for 10 min., and the pellet was macerated with liquid nitrogen, and homogenized (10:1 w/v) in 100 mM potassium phosphate buffer (pH 7.5) and 2.5 mM dithiothreitol and 5% (w/v) polyvinylpyrrolidone [38], always kept at 4°C. The homogenate was centrifuged at 10,000×g for 30 min., and the supernatant was divided into aliquots, and frozen at −80°C for subsequent enzyme analysis. The protein concentration was determined by the Bradford method [39], using BSA as standard.

Protein analysis by polyacrylamide gel electrophoresis (PAGE)

Electrophoresis was performed in gels containing 12% polyacrylamide with 4% stacking gel. For SOD-PAGE, SDS was eliminated. A current of 15 mA gel⁻¹ was applied for 3 h (SOD activity gel) at 4°C or 2 h (protein profile gel) at room temperature. Equal amounts of protein (20 μg) were applied to the gels. For SDS-PAGE, the gel was washed with distilled water, and incubated overnight in 0.05% Coomassie blue R-250 solution, at a ratio of 40:7:53 of methanol:acetic acid:water (v/v/v), and decolorized by successive washings with a solution, at a ratio of 40:7:53 of methanol:acetic acid:water (v/v/v) [40].

The SOD-PAGE activity was performed according to Beaucamp and Fridovich [41] and modified by Medicì et al. [42], in which the gels were washed in distilled water, and incubated in the dark for 30 min. in 50 mM potassium phosphate buffer (pH 7.8), containing 1 mM EDTA, 0.005 mM riboflavin, 0.1 mM nitroblue tetrazolium, and 0.3% N,N,N,N-tetramethylethylenediamine. To control the reaction, a unit of bovine liver SOD (Sigma) was used. The gels were exposed to white light and immersed in water until the development of the SOD bands.

Catalase (CAT) activity

CAT activity was determined according to Kraus et al. [43] in a solution containing 1 mM of potassium phosphate buffer 100 mM (pH 7.5) and 2.5 μL H₂O₂ (30% solution), and quantified in a spectrophotometer at 25°C. The reaction was initiated with the addition of 25 μL of protein extract, and the activity was determined by following the decomposition of H₂O₂ at 240 nm for 1 min.

GST activity

GST activity was measured in a solution containing 900 μL of potassium phosphate buffer 100 mM (pH 6.8), adding 25 μL of 1-chloro-2,4-dinitrobenzene (CDNB) 40 mM and 50 μL of reduced glutathione (GSH) 0.1 M, and incubated at 30°C [44]. The reaction was initiated with the addition of 25 μL of protein extract, and was monitored for 2 min. at 340 nm.

Analysis of lipids saturation

Bacterial strain was grown in 800 mL of LB and incubated at 37°C, at 200 rpm. After 12 h, the samples were centrifuged at 8,000×g for 5 min. at 4°C. The pellet was washed twice with PBS and divided into vials containing 50 mL with LB and LB plus 0.04 mM mesotrione, in triplicate, and the cultures were incubated at 37°C, at 200 rpm. After 12 h, lipid extraction was performed, as described by Bligh and Dyer [45], with modifications. The membrane lipids were analyzed by FTIR (Fourier Transform Infrared Spectroscopy) with transmittance at wavelengths from 400 to 4,000 cm⁻¹.

Experimental design and statistical analysis

To assess the saturation of lipids, the baseline of spectrums were corrected, and then processed by PCA (Principal Component Analyses) implemented in the Pirouette v. 4.0 software (Infometrix, Bothell, WA, USA). PCA was applied to separate the samples according to their FTIR spectra (1,400 to 5,200 cm⁻1). Therefore, the results obtained for each wavelength were plotted as columns and the samples as rows. Mean-Center was used as pre-treatment of the results.

Statistical analysis were conducted with three repetitions of each treatment for cell viability, MDA, GST and CAT experiments, which were performed in a completely random design. The significance of the observed differences was verified using a one-way analysis of variance (P<0.05). Analysis were made using R software version 3.0.1.

Results and Discussion

Mesotrione degrading capacity by E. coli DH5-α

E. coli is considered a model bacterium for study the physiology of prokaryotes [35]. Studies have been using the E. coli DH5-α strain as a recipient of genetic material, and it is one of the strains most frequently used as a tool in recombinant DNA technology [36,46–47]. Thus, we considered this strain as a laboratory, non-environmental bacterium, with no prior contact with herbicides.
The in vitro evaluation of the toxic effects of herbicides revealed a negative effect on the growth of strains of *E. coli*, particularly at higher doses [34], and also served as a model for the identification of genes and enzymatic activities involved in the antioxidative system in general [48–50]. Until this date, there are no studies involving herbicides degrading ability by non-environmental strains as *E. coli* DH5-α.

According to Batisson et al. [51], in high concentrations, mesotrione can alter the microbial community, selecting tolerant in 24 h and 18 h, respectively. Similarly, *E. coli* DH5-α have been described as capable to degrade mesotrione within 3 h and 10 h, respectively. Similarly, *P. ananatis* CCT 7673 [32] have been described as capable to degrade mesotrione without the presence of carbon [32].

In studies of mesotrione degradation in soil, it was observed that the highest rate of degradation of Callisto occurred with the application of 10× and 100× [33]. *P. ananatis* CCT 7673 (a strain isolated from water) was able to completely metabolize the herbicide mesotrione, but could not grow when in contact with the herbicide at high doses [32]. In our studies, *E. coli* DH5-α was able to toluene and degrade the herbicide in 10× and in a shorter incubation time. The tolerance exhibited by this bacterium to the herbicide may be explained by its rapid metabolism, since the degradation process takes place as soon as the strain is exposed to the xenobiotic, reducing the time of exposure of the bacterium to the chemical.

Studies of mesotrione degradation reported difficulty in finding specific genes for the degradation of this herbicide [23,43]. According to Pileggi et al. [32], the strains *E. coli* DH5-α, TOP 10, and K-12 have the ability to metabolize mesotrione. Considering that they are related to strains developed in the laboratory, with no prior contact with the herbicide, this fact may indicate low selective pressure for specific genes to degrade mesotrione.

Characterization of mesotrione herbicide as a stress agent

In order to determine if mesotrione damaged cells of *E. coli* DH5-α, its cellular viability was assessed under the same growth conditions as for protein extraction (Fig. 3). The data obtained revealed that the metabolic process was shown to initiate in the first hour of growth; therefore, the *E. coli* DH5-α strain was probably in contact with the whole herbicide for at least the initial growing of 30 min. During this period, a decrease in cell viability was observed in the treatments with the herbicide (MM and -CM), compared with the controls (MM and -C) (Fig. 3), indicating a toxic effect of the herbicide on the *E. coli* DH5-α.

During the periods of 3 h and 6 h, similar viability rates were verified for all treatments, which is probably due to the capacity of the nutritional support from MM provided to the bacterial cells.

Botelho et al. [34] analyzed many widely used commercial formulations of herbicides, and only paraquat decreased the growth of the *E. coli* ATCC 25922 strain. In the present study, field doses (1×) of mesotrione, which is considered less toxic than the commercial herbicide [22], decreased the viability of *E. coli* DH5-α within 30 min. in treatments with the herbicide (MM and -CM) (Fig. 3).

Balagüé et al. [53] used doses of 2 mM, 1 mM, 0.1 mM and 0.01 mM of the herbicide 2,4-D in spectrophotometer analysis, and verified growing inhibition of *E. coli* HB101 only at 2 mM, whilst in the present study, the growth capacity, analyzed by cellular viability, showed significant differences in *E. coli* DH5-α between control and mesotrione treatments in 30 min. evaluation (Fig. 3).

The malondialdehyde (MDA) rate has been used as an indicator of lipid peroxidation in other studies involving oxidative stress, such as that reported by Lima and Abdalla [54]. In the present study, *E. coli* DH5-α exhibited lower membrane damage, in the -CM treatment, within 30 min., while mesotrione still remained in the culture medium, (Fig. 4). Although cell viability was affected by the presence of mesotrione (Fig. 3), in the three analyzed times, the rates of MDA measured did not show an increased lipid peroxidation, in the -CM treatment. Nevertheless, in the MMM treatments, during all the periods analyzed, a statistically significant increase in MDA was observed, suggesting an imbalance in ROS production/ROS homeostasis, and consequently a toxic effect of the extra ROS was produced.

Influence of mesotrione on the level of lipids

Infrared spectrogram analysis showed changes in the structure of the membrane lipids of *E. coli* DH5-α in the presence of the herbicide mesotrione (Fig. 5). According to Böger et al. [55], an increase in the proportion of unsaturated fatty acids in bacterial cell membranes, makes them more susceptible to attack by ROS, and induces to higher rates of MDA production. These changes in membrane lipids occur from chloroacetanilide herbicides. A study

![Figure 3. Cellular viability of *E. coli* DH5-α in MM and in the MMM, -C and -CM treatments, during the periods of 30 min., 3 h and 6 h. LSD = 0.13 for all pairwise comparison. doi:10.1371/journal.pone.0099960.g003](image-url)
by Balangué et al. [53] with E. coli HB101 strain demonstrated that the toxicity of 2,4-D could reduce the fluidity of bacterial membrane, and consequently alter MDA results, as a system-wide response against ROS. Sánchez et al. [56] also reported similar findings, where the strain Klebsiella planticola DSZ, when in contact with ethanol or the herbicide simazine, exhibited a decrease in the saturation of membrane lipids, altering the rate of selective permeability, possibly as a defense system.

In the present study, such a change in the composition of the membrane lipids of E. coli DH5-α may explain the lower rate of MDA in the treatments without the presence of carbon and with mesotrione (Fig. 4), as shown by data from cell viability (Fig. 3), in which the presence of mesotrione caused damage to bacteria in a time of 30 min. Apart from defense against ROS, the change in selective permeability may have prevented the entrance of mesotrione, with increased saturation of membrane lipids, characterizing a defense system against xenobiotics.

Involvement of antioxidant enzymes in the defense and degradation of mesotrione

By superoxide dismutase gel analysis, up to 6 distinct isoenzymes (Fig. 6, lane 3) were observed among the treatments used (Fig. 6). These isoenzymes were not necessarily present in all treatments, but the majority of the SOD activity detected could be accounted to SOD I and SOD IV isoenzymes. A larger number of bands were always observed when E. coli DH5-α was in MM (Fig. 6, lane 3) and MMM (Fig. 6, lane 6), at 6 h of exposure/growth. The higher SOD activity observed, under such conditions, and based on the higher intensity of the SOD bands, could be attributed to the increased number of visible SOD bands, and also to the higher activity of SOD I and SOD IV isoenzymes, which together clearly accounted for the majority of the SOD activity in E. coli DH5-α. Yet, it is likely that this difference on band intensity was due to the incubation time of E. coli DH5-α, and consequently occurred an increase of superoxide radical production. As shown in the treatment with carbon (MM and MMM), as longer the growing time, the higher was SOD activity. However, this was not observed in the treatment without carbon (-CM). Ongoing research is classifying the distinct SOD isoenzymes, as proposed by Azevedo et al. [57], detecting which shall be important in future studies, since they may be located in distinct cell compartments, and consequently allow to link an increase in superoxide in a specific organelle to an specific increase of one or another particular SOD isoenzyme.

An increase in CAT activity was observed, depending on the exposure time in the culture media with the presence of carbon (MMM and MM) (Fig. 7), which was probably due to the adaptation and growth of the strain in the mineral medium. However, there were no differences in relation to the presence of mesotrione. In strains of E. coli, depending on the growth phase, different isoforms can act, which have regulatory pathways that are activated independently [58]. This may have influenced the

Figure 4. MDA levels in E. coli DH5-α in MM, and in the MMM, -C and -CM treatments, during periods of 30 min., 3 h and 6 h, respectively. LSD = 0.18 for all pairwise comparison.

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Figure 5. Analysis of lipids of E. coli DH5-α using FTIR and principal component analysis. Points M2 and M5: control without mesotrione (LB), in duplicate. Points M1, M3 and M4: treatment with mesotrione (LB+0.04 mM mesotrione) in triplicate. Point B2: negative control, Bacillus sp. in the absence of mesotrione.

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CAT activity response and also the potential participation of other peroxidases in the stress response.

In the culture media without carbon (-C and -CM), despite showing a significantly lower total CAT enzymatic activity, in comparison with the media with carbon, the activity of CAT was higher in the media with mesotrione, except in 3 h exposure period (Fig. 7). In the period of 30 min., during which the complete degradation of the herbicide had not yet occurred, and mesotrione appeared as the only carbon source, the amount of hydrogen peroxide may have increased in response to the xenobiotic itself, stimulating CAT activity, which is notable when compared to the control without carbon source (-C).

In relation to *E. coli* DH5-α, GST activity was higher in the 30 min. exposure treatments in the presence of mesotrione, compared to medium without the herbicide. However, in the 3 h period, no differences occurred in the activities of GST (Fig. 8). There are reports about metabolization of triketone herbicides (such as mesotrione) by influence of cytochrome P450 in plants [24–25]. According to Barrett [24], P450 is essential for the biodegradation of at least six families of herbicides used in maize culture. Furthermore, both the P450 as GST are involved in cellular defense and detoxification of these herbicides in maize and rice [26].

The involvement of the cytochrome P450 enzyme system in degradation of atrazine and herbicides EPTC (-Ethyl Dipro-pyliothiocarbamate) was observed in bacteria [59–60], but at a very slow rate [61]. A P450 like enzyme, synthesized by the *syg* gene, is involved only in the reduction pathway of paraquat in *E. coli*, not in the degradation [29], thereby changing the mode of action of this herbicide [62].

On the other hand, GST enzymes have been characterized in plants and microorganisms, as mechanisms aiding the metabolism of herbicides and other toxic products, catalyzing the conjugation of glutathione with the herbicide and marking the compound to be degraded [27–28]. However, there is a great diversity in this enzyme, with various functions still unknown [21,63–64].

**Conclusions**

This is the first report showing that *Escherichia coli* DH5-α, which is considered a non environmental strain, was able to degrade mesotrione without previous exposure to the herbicide. The process of degradation took only 3 h, being the lowest degradation time reported until now. Previous articles tried to discover a gene responsible for mesotrione degradation, without success. In this manuscript, we describe the involvement of GST in herbicide degradation, as part of a more complex response system to mesotrione. Nevertheless, it cannot be ruled out that other systems may also be involved cooperatively or independently, such as other peroxidases.

Mesotrione was identified as an oxidative stress agent by the involvement of GST in herbicide degradation, changes in membrane lipids saturation, preventing membrane cell peroxidation, and differences in cellular viability. In this case, MDA did not indicate the occurrence of cellular damage by oxidative stress, but its reduction was related to changes on lipid structure in response to the herbicide. To our knowledge, this manuscript is the first report on the characterization of mesotrione as an oxidative stress agent in bacteria. Nevertheless, we described *Escherichia coli* DH5-α as a tolerant strain and capable of growing in the presence of the herbicide at concentrations normally used in the environment by agricultural management, even without previous contact with the herbicide. We consider that *E. coli* strains can manage to adapt to
the presence of new synthesized toxic molecules in its environment through non-specific mechanisms of tolerance, regarding to non-specific anti stress enzymes involved with degradation, as GST, and changes in structure of lipid membrane, perhaps preventing the entrance of the herbicide in the cell in its toxic configuration. *E. coli* DH5-α, already a model for different studies in bacterial metabolism and adaptation, can also be used for the study of other enzymatic and structural systems related to herbicides tolerance and adaptation in contaminated environments, through phenotypic plasticity of those systems.

### References

1. Dayan FE, Cantrell CL, Duke SO (2009) Natural products in crop protection. Bioorg Med Chem 17: 4022–4034.
2. Armas EDD, Monteiro RTR, Antunes PM, Santos MAPFD, Camargo PBD, et al. (2007) Diagnóstico espac¸o-temporal da ocorreˆncia de herbicidas nas a´guas superficiais e sedimentos do Rio Corumbataı´ e principais afluentes. Quı´m Nova 30: 1119–1127.
3. Sandi DC, Morris CE, Dratz EA, Pilgeram A (2004) Pesticides industry sales and usage: 2000 and 2001 market estimate. US Environmental Protection Agency 1–33.
4. Kiely T, Donaldson D, Grube A (2004) Pesticides industry sales and usage: 2000 and 2001 market estimate. US Environmental Protection Agency 1–33.
5. Jiang L, Huang J, Liang L, Zheng PY, Yang H (2008) Mobility of prometryne in soil as affected by dissolved organic matter. J Agric Food Chem 56: 11933–11940.
6. Lopes-Perezi GC, Arias-Esteviz M, Lopez-Priaga E, Soto-Gonzalez B, Cancho-Grande B, et al. (2006) Dynamics of pesticides in potato crops. J Agric Food Chem 54: 1797–1803.
7. Martins PF, Martínez CO, Carvalho RF, Grata ˜o PL, Carvalho G, Tezotto T, et al. (2011) Selection of microorganisms degrading s-metolachlor herbicide. Braz Arch Biol Technol 50: 153–159.
8. Sharma D, Nagpal A, Pakade YB, Katnoria JK (2010) Estimation of organophosphorus pesticide residues in fruits and vegetables: A review. Talanta 82: 1077–1089.
9. Cycon M, Markovskiz A, Piotrowska-Seget Z (2013) Structural and functional diversity of bacterial community in soil treated with the herbicide napropamide estimated by the DGE, CLIP and r/K-strategy approaches. Appl Soil Ecol 72: 242–250.
10. Hernandez M, Jia Z, Conrad R, Seeger M (2011) Simazine application inhibits enzymatic and structural systems related to herbicides tolerance, regarding to non-specific anti stress enzymes involved with degradation, as GST, and changes in structure of lipid membrane, perhaps preventing the entrance of the herbicide in the cell in its toxic configuration. *E. coli* DH5-α, already a model for different studies in bacterial metabolism and adaptation, can also be used for the study of other enzymatic and structural systems related to herbicides tolerance and adaptation in contaminated environments, through phenotypic plasticity of those systems.

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Conceived and designed the experiments: LRO MND FLB SAVP RAA MJS MP. Performed the experiments: LRO MND FLB SAVP RAA MJS MP. Analyzed the data: LRO MND FLB AAFZ IMD SAVP RAA MJS MP. Contributed reagents/materials/analysis tools: FLB AAFZ IMD SAVP RAA MJS MP. Wrote the paper: LRO MND FLB AAFZ IMD SAVP RAA MJS MP.
31. Duran S, Sancelme M, Bese-Hoggan P, Combrouie B (2010) Biodegradation pathway of mesotrolone: complementarities of NMR, LC-NMR and LC-MS for qualitative and quantitative metabolic profiling. Chemosphere 81: 372–380.

32. Paleggi M, Paleggi SAV, Olchanedi KS, Silva PAG, Gonzalez JMM, et al. (2012) Isolation of mesotrolone-degrading bacteria from aquatic environments in Brazil. Chemosphere 86: 1127–1132.

33. Crouzet O, Batiston I, Bese-Hoggan P, Boumey F, Badot C, et al. (2010) Response of soil microbial communities to the herbicide mesotrolone: a dose-effect microcosm approach. Soil Biol Biochem 42: 193–202.

34. Botelho RG, Froes CM, Santos JB (2012) Toxicity of herbicides on Funct Plant Biol 61: 337–344.

35. Williams JA, Carnes AE, Hodgson CP (2009) Plasmid DNA vaccine vector Escherichia coli. J Coll Int Sci 275: 177–182.

36. Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125: 189–196.

37. Kraus TE, Mckersie BD, Fletcher RA (1995) Paclobutrazol-induced tolerance of tomato (Lycopersicon esculentum cv. Micro-Tom) plants to cadmium-induced stress. Ann Appl Biol 125: 311–323.

38. Williams JA, Carnes AE, Hodgson CP (2009) Plasmid DNA vaccine vector Escherichia coli. J Coll Int Sci 275: 177–182.

39. Heath RL, Parker L (1968) Photoperoxidation in isolated chloroplasts. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125: 189–196.

40. Grata˜o PL, Monteiro CC, Antunes AM, Peres LEP, Azevedo RA (2008) Effects of metal contamination on a consortium of fish from a harbor. Braz J Biol 72: 141–146.

41. Beauchamp C, Fridovich I (1971) Superoxide Dismutase: improved assays and positive control. Anal Biochem 44: 276–287.

42. Shao ZQ, Behki R (1995) Cloning of the genes for degradation of the herbicides EPTC (a-ethyl dipropylphiocarbamate) and atrazine by Rhodococcus sp. strain N86/21. Appl Environ Microbiol 61: 2056–2060.

43. Bo¨ger P, Matthes B, Schmadl F (2000) Towards the primary target of chloroacetamides - new findings pave the way. Pest Manag Sci 56: 497–508.

44. Ma XX, Jiang YL, He YX, Bao R, Chen Y, et al. (2009) Structures of yeast glutathione S-transferase Gtt2 reveal a new catalytic type of GST family. EMBO J 28: 3487–3493.

45. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917.

46. Jung IL, Kim IG (2003) Transcription of ahpC, katG, and katE genes in Escherichia coli is regulated by polyamines: polyamine-deficient mutant sensitive to H2O2-induced oxidative damage. Biochem Biophys Res Commun 301: 915–922.

47. Crouzet O, Batisson I, Besse-Hoggan P, Bonnemoy F, Bardot C, et al. (2010) Biodegradation of mesotrione: complementarities of NMR, LC-NMR and LC-MS for qualitative and quantitative metabolic profiling. Chemosphere 81: 372–380.

48. Cummins I, Andon NL, Haynes PA, Park M, Fisher WH, et al. (2011) Multiple roles for plant glutathione transferases in xenobiotic detoxification. Drug Metab Rev 43: 266–280.