Universidade de Aveiro Departamento de Biologia
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Diana Rita Silva Matos

Shedding light on the effect of saturated aldehydes and alcohols in *Rhizobium* cells challenged by cadmium: growth and biochemistry

Elucidar os efeitos de aldeídos e álcoois saturados em células de *Rhizobium* expostas a cádmio: crescimento e resposta bioquímica

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Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em eco-toxicologia e análise de risco, realizada sob a orientação científica da Doutora Etelvina Figueira, Professora Auxiliar do Departamento de Biologia da Universidade de Aveiro e coorientação científica da doutora Adília O. Pires, Investigadora Auxiliar do Departamento de Biologia da Universidade de Aveiro.

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agradecimentos

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palavras-chave Rizóbio; cádmio; voláteis; aldeídos; álcoois; crescimento; parâmetros bioquímicos

resumo

Os rizóbios são bactérias do solo que têm um papel agronómico e ambiental significativo, sendo contribuidores decisivos para a fertilidade do solo. No entanto, estes microrganismos são afetados por stresses ambientais como a contaminação por cádmio, o que altera o metabolismo dos organismos, incluindo o volatiloma (conjunto de metabolitos sintetizados por um organismo) bacteriano. Dois grupos de compostos que sofrem alteração em células de Rizóbio expostas ao cádmio são os aldeídos e os álcoois saturados, contudo é pouco conhecido o efeito destes compostos nas células bacterianas. Nesta tese são estudados os efeitos de diferentes aldeídos (hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal e tridecanal) e álcoois (hexanol, heptanol, octanol e nonanol) separadamente ou em combinação com cádmio no crescimento e na bioquímica de células de *Rhizobium* (estirpe E20-8).

Os resultados mostram que aldeídos menores (com 6 a 10 carbonos) reduziram a peroxidação lipídica (37% a 50%), enquanto aldeídos maiores (com 11 a 13 carbonos) aumentaram a peroxidação lipídica ligeiramente (20% e 30%) ou significativamente (>70%), evidenciando a função protetora dos aldeídos menores e desestabilizadora dos maiores nas membranas. As enzimas com atividade antioxidante como a superóxido dismutase diminuiu na presença dos aldeídos menores e aumentou na presença dos maiores, mostrando a ativação de mecanismos antioxidantes em células expostas a aldeídos maiores. Na maioria das condições testadas a exposição aos diferentes aldeídos não influenciou o crescimento das células do *Rhizobium*, tanto na presença como na ausência de Cd, contudo a exposição a 1mM de decanal e 10 μM tridecanal causaram um aumento significativo no crescimento na condição de Cd.

A comparação dos efeitos de aldeídos e dos respetivos álcoois conjugados em células de *Rhizobium* expostas ou não a Cd permite elucidar qual dos dois tipos de compostos apresenta maior toxicidade para as células. Os resultados evidenciaram a maior toxicidade da maioria dos aldeídos comparativamente ao respetivo álcool conjugado na presença de Cd, sugerindo que a redução de aldeídos a álcoois pode ser um mecanismo efetivo de restrição da toxicidade de aldeídos.

Esta tese apresenta informação nova que ajuda a esclarecer as alterações que ocorrem em células bacterianas expostas a Cd e os mecanismos induzidos pelas células para minimizar essa mesma toxicidade. O contributo é especialmente relevante na influência que estes compostos possam ter em contextos de proteção das comunidades bacterianas sob stresse ambiental.

Keywords

Rhizobium; cadmium; volatiles; aldeydes; alcohols; growth; biochemical endpoints

Abstract

Rhizobia are soil bacteria that play significant agronomic and environmental roles and are decisive contributors to soil fertility. However, these microorganisms are affected by environmental stresses such as cadmium contamination, which alters the metabolism of organisms, including bacterial volatilome (a set of metabolites synthesized by an organism). Two groups of compounds that change in cadmium-exposed rhizobia cells are saturated aldehydes and alcohols, yet the effect of these compounds on bacterial cells is poorly known.

In this thesis the effects of different aldehydes (hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal) and alcohols (hexanol, heptanol, octanol and nonanol) separately or in combination with cadmium on growth and biochemistry of *Rhizobium* cells (strain E20-8) are evaluated.

Results show that smaller aldehydes (6 to 10 carbons) reduced lipid peroxidation (37% to 50%), while larger aldehydes (11 to 13 carbons) increased it slightly (20% and 30%) or significantly (> 70%), showing the protective function of the smaller aldehydes and the destabilizing effect of the largest ones in membranes. Enzymes with antioxidant activity such as superoxide dismutase decreased in the presence of smaller aldehydes and increased in the presence of larger ones, showing the activation of antioxidant mechanisms in cells exposed to larger aldehydes. Exposure to different aldehydes did not influence *Rhizobium* cell growth in most of the conditions tested, either in the presence or absence of Cd. However, exposure to 1mM decanal and 10 μM tridecanal caused a significant increase in Cd growth.

The comparison of aldehydes with their conjugated alcohols effects on *Rhizobium* cells exposed or not to Cd allowed to elucidate which of the two types of compounds was more toxic to cells. Results showed the higher toxicity of most aldehydes compared to their conjugated alcohol in the presence of Cd, suggesting that reduction of aldehydes to alcohols may be an effective mechanism of aldehyde toxicity restriction.

This thesis presents new information that helps clarify the changes occurring in bacterial cells exposed to Cd and the cell-induced mechanisms to minimize this toxicity. The contribution is especially relevant in influence that these compounds may have in the protection of bacterial communities under environmental stress contexts.

Index

Abbreviations

Cd - Cadmium

VOCs - Volatile organic compounds

YMA - Yeast mannitol agar

LPO - Lipid peroxidation

TCA - Trichloroacetic acid

SOD - Superoxide dismutase

GSTs - Glutathione S-transferases

GPx - Glutathione peroxidases

PC - Protein carbonylation

EDTA - Ethylenediaminetetraacetic acid disodium salt dehydrate

PVP - Polyvinylpyrrolidone

DTT - Dithiothreitol

BSA - Bovine serum albumin

TBARS - Thiobarbituric acid reactive substances

MDA - Malondialdehyde

DNPH - 2, 4-Dinitrophenylhydrazine

NBT - Nitro blue tetrazolium

GSH - Reduced glutathione

CDNB - 1-chloro-2,4-dinitrobenzene

PCO - Principal coordinates method

ROS - Reactive oxygen species

PUFAs - Polyunsaturated fatty acids

Lys - Lysine residues

N_2 - Nitrogen

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Chapter 1 – Introduction

1.1. Relevance of *Rhizobium*

Soil microorganisms provide important services in soil fertility and ecosystem productivity, both in agricultural and natural areas.

Rhizobacteria live in close association with plant roots and some of them, such as *Rhizobium leguminosarum* (fig. 1), are able to establish a well-known endosymbiotic relationship with plants belonging to the Fabaceae family [1, 2]. This symbiosis allows the conversion of diatomic nitrogen into nitrogen forms that can be used by plants [1]. Persistence of N_2-fixing microorganisms in contaminated soils may increase the resilience of communities from contaminated sites, thus decreasing the impact on density and biodiversity of these communities [3].

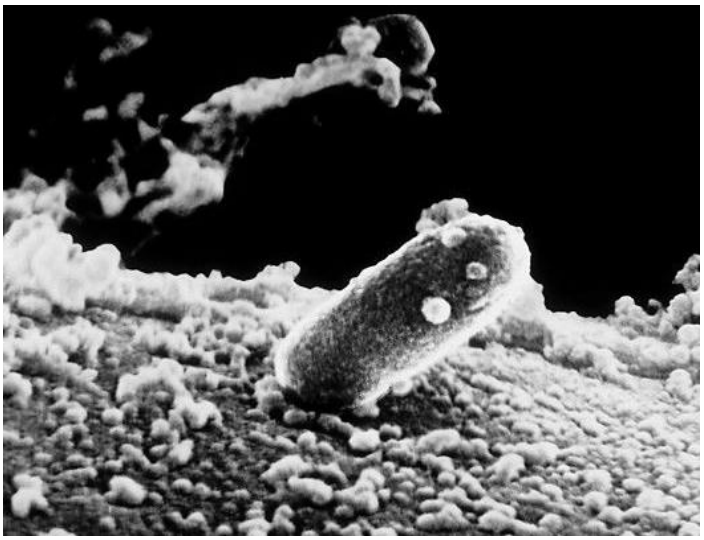


Figure 1 - Scanning electron micrograph (SEM) of *Rhizobium leguminosarum*, on the root hair of a pea plant, *Pisum sativum*. (from Burgess, 2010).

The genome sequence of *Rhizobium leguminosarum*, a species that nodulates, among other legumes, *Pisum sativum*, was published almost ten years ago [4]. The genome is surprisingly big for a bacteria, maybe due to the accumulation of genetic material that can increase the organism's plasticity so they can face the dynamic nature of soil and its dynamic substrates [4].

When in free-living form, among the soil microbiome, rhizobia can also play an important role in plant growth promotion, benefiting both legume and non-legume plants [5]. This positive effect is achieved by the ability to solubilize phosphates, produce phytohormones (auxins, cytokinins and gibberellins) and siderophores (that form complexes with metals such as Fe, Cd, Zn, Cu, Pb and

Al), synthesize compounds with antimicrobial activity against pathogens and enhance the activity of plant enzymes, namely proteases and lipases [5, 6].

Persistence of *Rhizobium* in the soil and effectiveness of nodulation may be affected by a number of environmental factors, such as drought [7, 8], salinization [8, 9] or contamination by non-essential metallic elements [8, 10, 11]. Regarding contamination by nonessential metals such as cadmium, intensive farming accounts for a big fraction [8]. The use of phosphate based fertilizers is a major input of Cd in soils, since these fertilizers are produced from phosphate rocks, which, depending on their origin, might be rich in Cd [8].

1.2. Cadmium stress

Cadmium is classified as the seventh more toxic substance on the 2015 Priority List of Hazardous Substances by the Agency for Toxic Substances and Disease Registry [12]. Therefore, even at low concentrations the potential to affect soil communities is high, so Cd imposes a serious threat to organisms [13], because of the high affinity for sulfhydryl groups, and the ability to replace metal cofactors in metalloenzymes, leading to the direct inactivation of important proteins of cell metabolism [14]. The Cd displacement of redox active metals can inhibit electron transport chains and lead to reactive oxygen species (ROS) burst [15]. ROS interact with lipids, proteins and DNA, causing lipid peroxidation (LPO), protein carbonylation (PC) and DNA methylation [15], affecting gene and protein expression, membrane fluidity and permeability and enzymes activity, thus compromising cell homeostasis [15]. Cellular metabolism and key enzymes can be disrupted by ROS, and in order to survive cells must trigger mechanisms to alleviate Cd toxicity.

To reduce Cd effects, cells possess Cd-chelating and ROS scavenging mechanisms that include low molecular weight antioxidant compounds such as glutathione (GSH). Since this mechanism is not always efficient, other mechanisms are triggered, such as volatile organic metabolites [1] (fig. 2) and enzymatic mechanisms, such as superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferases (GSTs) (fig. 2) [16, 17]. GSTs directly neutralize Cd toxicity by catalyzing the conjugation of GSH to Cd ions, increasing the formation of Cd-GSH conjugates [3, 17].

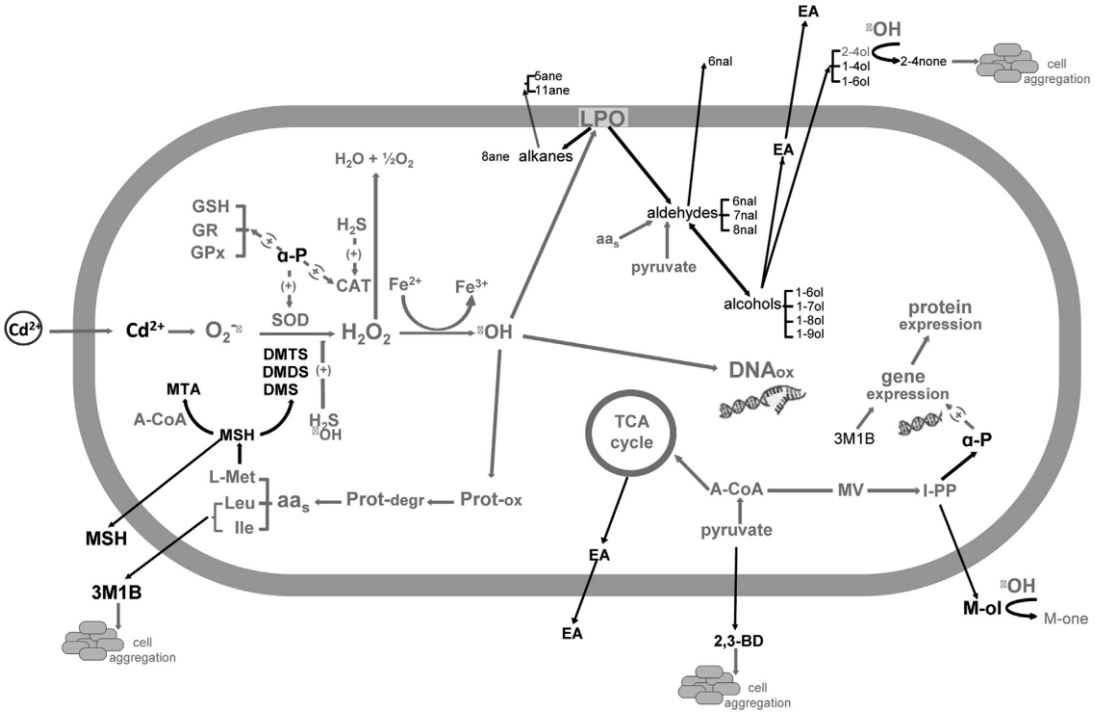


Fig. 2 - Volatile metabolites (VOCs) involved in *Rhizobium* sp. strain E20-8 response to the stress induced by cadmium. The metabolic pathways and possible effects are represented (in Cardoso, 2014).

1.3. Volatile organic compounds: Aldehydes and alcohols

Volatile organic compounds (VOCs) encompass various chemical classes, including low molecular weight fatty acids and their derivatives (hydrocarbons, alcohols, aldehydes and ketones), terpenoids, aromatic compounds, nitrogen containing compounds, and volatile sulphur compounds [18].

Bacteria release a wide variety of biologically active airborne VOCs that can diffuse. Most microbial volatiles are considered as side-products of primary and secondary metabolism [19]. The underlying biosynthetic pathways are aerobic, heterotrophic carbon metabolism, fermentation, amino-acid catabolism, terpenoid biosynthesis, fatty acid degradation and sulphur reduction [20]. Although VOC production is considered a general phenomenon of bacteria [21] the interference of these compounds with cell metabolism is poorly understood.

In this thesis two chemical classes: aldehydes and alcohols are addressed, specifically four saturated aldehydes and the corresponding alcohols (fig. 3) and in addition four more saturated aldehydes (fig. 4).

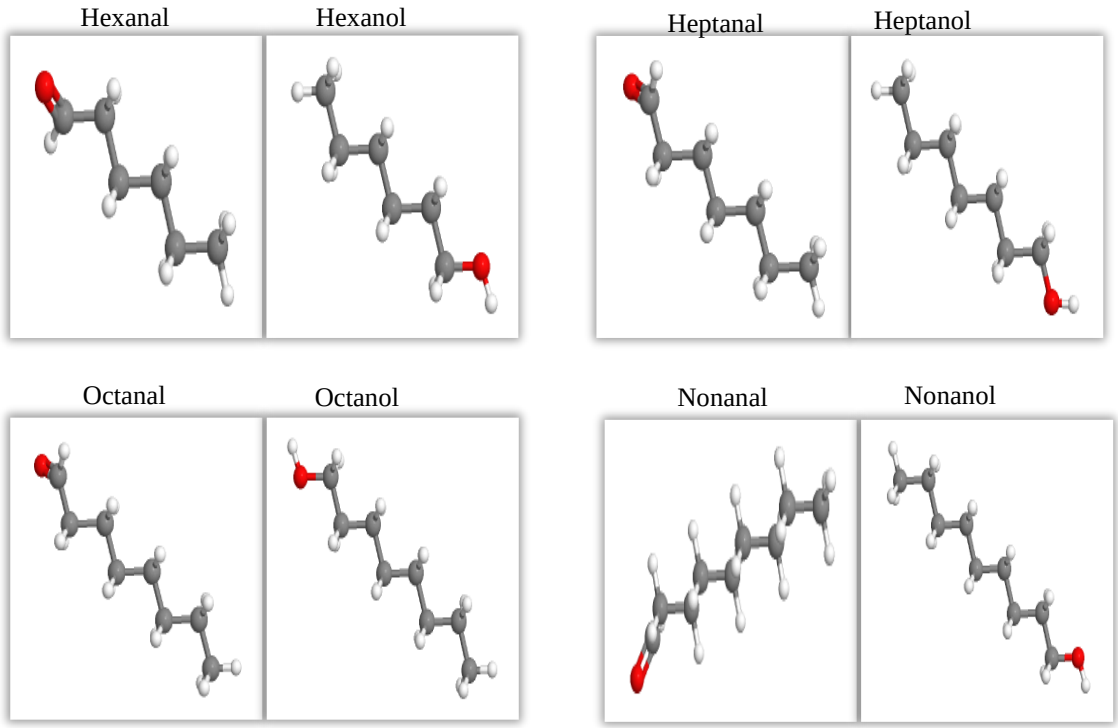


Fig. 3 – 3D chemical structure of hexanal, hexanol, heptanal, heptanol, octanal, octanol, nonanal and nonanol.

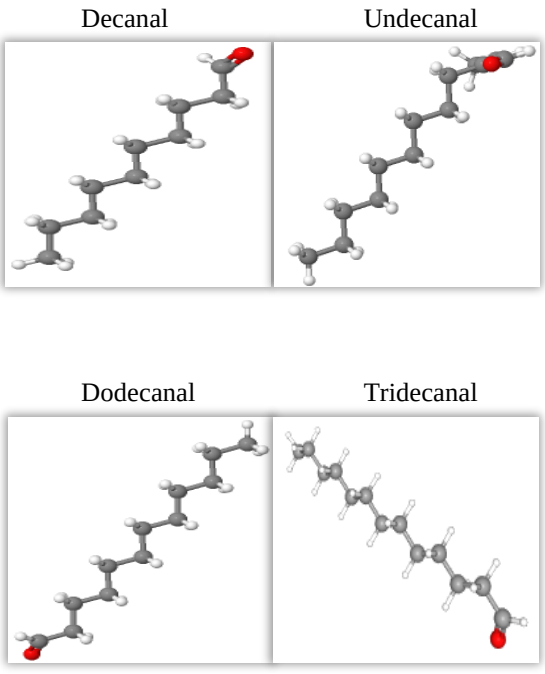


Fig. 4 – 3D chemical structure of decanal, undecanal, dodecanal and tridecanal .

Aldehydes are highly reactive molecules that are intermediates or products involved in a broad spectrum of physiological, biological and pharmacological processes [22]. Aldehydes are

generated from chemically diverse endogenous and exogenous precursors and aldehyde-mediated effects vary from homeostatic and therapeutic to cytotoxic, and genotoxic. One of the most important pathways for aldehyde metabolism is their oxidation to carboxylic acids by aldehyde dehydrogenases (ALDHs) [23].

In order to deal with aldehydes toxicity, mammals have evolved a battery of enzymes which convert these compounds to less reactive chemical species. The main reactions of aldehydes are the adduction with glutathione (GSH), which can either occur spontaneously or be catalysed by glutathione S-transferases (GSTs), the reduction to alcohols by aldo–ketoreductases (AKRs) or alcohol dehydrogenase and the oxidation to acids by aldehyde dehydrogenases (fig. 5) [24].

The alcohol dehydrogenase (ADH) and the aldehyde dehydrogenase (ALDH) are also present in bacteria. Membrane-bound alcohol dehydrogenase (ADHa) of *Gluconacetobacter diazotrophicus*, is an enzyme which is able to use primary alcohols (C2–C6) and its respective aldehydes as alternate substrates [25].

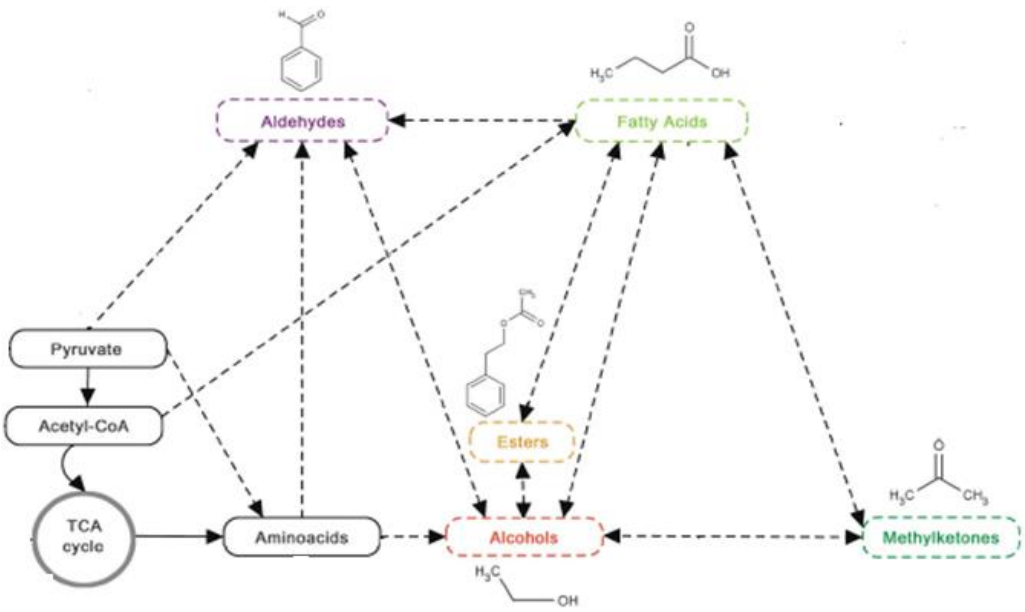


Figure 5 - Main metabolic pathways for the production of microbial volatiles. Volatiles are depicted in colored dashed rectangles indicating different chemical classes (adapted from Schmidt, 2015).

The ALDH superfamily catalyzes the oxidation of numerous aldehyde substrates and plays a particularly critical role in the cellular protection against toxic species, as evidenced by the fact that mutations and polymorphisms in ALDH genes (leading to perturbations in aldehyde metabolism) are the molecular basis of several disease states and metabolic anomalies [24] .

The metabolism of amino acids, carbohydrates, lipids, biogenic amines, vitamins, and steroids generates endogenous aldehydes, also the biotransformation of a large number of drugs and

environmental agents generates aldehydes. Aldehyde toxicity is mainly due to the alterations of several cell functions, which mostly depend on the formation of covalent adducts with cellular proteins [26]. Thanks to their amphiphilic nature, aldehydes can easily diffuse across membranes and can covalently modify any protein in the cytoplasm and nucleus, far from their site of origin [27]. Most of the available information on the effects of aldehydes is on mammalian cells, and information available for bacterial cells is virtually non-existent. Andersen and Ingo [28] reported that alcohols interact with lipid bilayers and present a chain-length-dependent lipid bilayer-alcohol interaction [28]. Moreover, alcohols can also alter protein function through the ability to directly interact with proteins. The efficacy of alcohols of various chain lengths tends to exhibit a so-called cutoff effect (i.e., increasing potency with increased chain length, which eventually levels off) [28]. The information regarding interaction of alcohols with cells and cell components is also mainly on animal cells.

1.4. Aims of dissertation

Since aldehydes and alcohols are compounds present in *Rhizobium* metabolism, this dissertation intends to fill this gap and increase the knowledge about the effects of these compounds on bacterial cells. Moreover, since saturated aldehydes and alcohols increase in cells exposed to metal stress [1] and given their toxicity towards cells, this dissertation also aims to bring information on the effects and mode of action of these compounds in metal challenged cells. To achieve this, we analyzed growth and biochemical endpoints related to oxidative stress such as damage (lipid peroxidation and protein carbonylation), protein content and enzymatic activity (superoxide dismutase, glutathione-s-transferase and glutathione peroxidase. These parameters allowed us to understand the physiological and biochemical status of Rhizobium cells exposed to the compounds tested.

The effects of four saturated aldehydes and their corresponding alcohols in *Rhizobium* strain E20-8 will be addressed in chapter 3. The effects of different saturated aldehydes (C6 to C13) in *Rhizobium* strain E20-8 will be analyzed in chapter 4.

Chapter 2- Material and methods

2.1. Strain

Rhizobium sp. strain E20-8 (partial 16S rRNA sequence Genbank accession number KY491644), previously isolated from root nodules of *Pisum sativum* L. plants grown in a non-contaminated field in Southern Portugal and reported as tolerant to Cd [8, 9] was used in this work.

2.2. Growth curve in the presence of Cd

After an initial inoculum from a stock culture, 18 colonies were inoculated in one side of a split plate containing yeast extract mannitol agar (YMA) [21]. Plates were incubated at 26 °C during 4 days, with 3 independent replicates. The growth curve was obtained by measuring colonies diameter at 12h intervals, during 96h. Results were used to construct a growth curve. Based on the growth curve, 60h growth was chosen for subsequent work as colonies were in full logarithmic growth. After an initial inoculum from a stock culture, 18 colonies, from a stock culture, were inoculated in plates containing YMA supplemented with 0 μM; 50 μM; 100 μM; 150 μM; 200 μM; 300 μM; 400 μM; 600 μM $CdCl_2$. Plates were incubated at 26 °C, during 60 h, with 3 independent replicates to determine growth inhibition and estimate IC_{50} (107.54 μM) (Fig. S1). Consequently, for further work 100 μM Cd was used.

2.3. Chemicals

High-purity VOCs (>99%) were purchased from Sigma-Aldrich. The chemicals used are the aldehydes and alcohols which increased in *Rhizobium leguminosarum* strain E20-8 exposed to Cd compared to control conditions [1]. Aldehydes, alcohols and each compound were expressed as an acronym: AL (aldehydes); OL (alcohols); 6-al (hexanal); 6-ol (hexanol); 7-al (heptanal); 7-ol (heptanol); 8-al (octanal); 8-ol (octanol); 9-al (nonanal); 9-ol (nonanol); 10-al (decanal); 11-al (undecanal); 12-al (dodecanal); 13-al (tridecanal).

2.4. Cell culture and exposure to Cd and VOCs

To perform the experiments on the effect of aldehydes and the corresponding alcohols on *Rhizobium* cells response to Cd exposure, the I-plate method was used [21] with some modifications. The range of concentrations used was established taking into account the study of Kim et al. [21]. The bacterial colonies were harvested with a sterile spatula. The five colonies growing on the same I-plate were pooled into a microtube, weighed and stored at -80 °C. The Cd concentration used imposed a growth inhibition around 50%. Aldehydes and alcohols stock solutions were prepared in 70% ethanol. Ethanol concentration used was confirmed to have no effect on rhizobia growth.

Twelve colonies were inoculated on one side of the plate containing yeast extract mannitol agar (YMA) (control condition) or YMA supplemented with 100 µM $CdCl_2$ (stress condition); on the other side of the I plate, 10 µL of each chemical at different concentrations was applied to a sterile paper disk (Ø= 0.5 cm). Plates were incubated at 26 °C, during 60 h, with 3 independent replicates for each condition, with a total of 3 replicates x 2 Cd concentrations (0 and 100 µM) x 6 chemical concentrations (0 nM, 1nM, 100 nM, 10 µM, 1 mM and 100 mM) x 8 compounds.

2.5. Biochemical parameters

Frozen cells were suspended in specific extraction buffers, disrupted by sonication using an ultrasonic homogenizer U 200 S Control (IKA-WERKE), while keeping tubes in an ice bath, and centrifuged at 12,000×g for 10 min at 4 °C. For lipid peroxidation (LPO), samples were extracted with 20% (v/v) trichloroacetic acid (TCA). For superoxide dismutase (SOD), glutathione S-transferases (GSTs), glutathione peroxidases (GPx), protein carbonylation (PC) and total soluble protein, sodium phosphate buffer (50mM sodium dihydrogen phosphate monohydrate; 50 mM disodium hydrogen phosphate dihydrate; 1mM ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA); 1% (v/v) Triton X-100; 1% (v/v) polyvinylpyrrolidone (PVP); 1mM dithiothreitol (DTT), pH 7.0) was used.

Total soluble protein content was determined according to the Biuret method [29], using bovine serum albumin (BSA) as standard (0-40 mg/mL). The absorbance was read at 540 nm. Results were expressed in mg of protein per g of bacteria (mg g^{-1}).

Lipid peroxidation (LPO) was measured by quantification of thiobarbituric acid reactive substances (TBARS), according to the method of Buege and Aust [30]. TBARS were quantified spectrophotometrically at 532 nm and calculated using the molar extinction coefficient of MDA ($\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$). Results were expressed in nmol of MDA equivalents per g of bacteria (nmol g^{-1}).

Protein carbonylation (PC) was measured by quantification of carbonyl groups (CG), according to the DNPH alkaline method described by Mesquita et al. [31] with modifications [32]. The amount of CG was quantified spectrophotometrically at 450 nm ($\varepsilon = 22.308\ mM^{-1}\ cm^{-1}$) and results were expressed in µmol of CG per g of bacteria (µmol g^{-1}).

Superoxide dismutase (SOD) activity was determined based on the method of Beauchamp and Fridovich [33] by the reaction of nitro blue tetrazolium (NBT) with superoxide radicals to form NBT diformazan. Absorbance was measured at 560 nm after 20 minutes of. One unit of enzyme activity (U) corresponds to a 50% reduction of NBT. Results were expressed in milliunits (mU) per g of bacteria (mU g^{-1}).

Glutathione peroxidase (GPx) activity was determined based on the method of Paglia and Valentine [34] with cumene hydroperoxide as substrate and using the glutathione reductase coupled assay to monitor the oxidation of glutathione (GSH). NADPH was added and the absorbance was immediately read at 340 nm, with continuous reading at 15 s intervals over 20 minutes. GPx activity was expressed in mU per g of bacteria (mU g^{-1}).

Glutathione S-transferases (GSTs) activity was determined following an adaptation of the method described by Habig et al. [35]. These enzymes catalyze the conjugation reaction of GSH with electrophilic substrates such as 1-chloro-2,4-dinitrobenzene (CDNB) [17]. GSTs activity was measured spectrophotometrically at 340 nm (ε = 9.6 mM^{-1} cm^{-1}) at intervals of 15 s during 20 min. The enzymatic activity was expressed in mU per g of bacteria (mU g^{-1}).

2.6. Data analysis

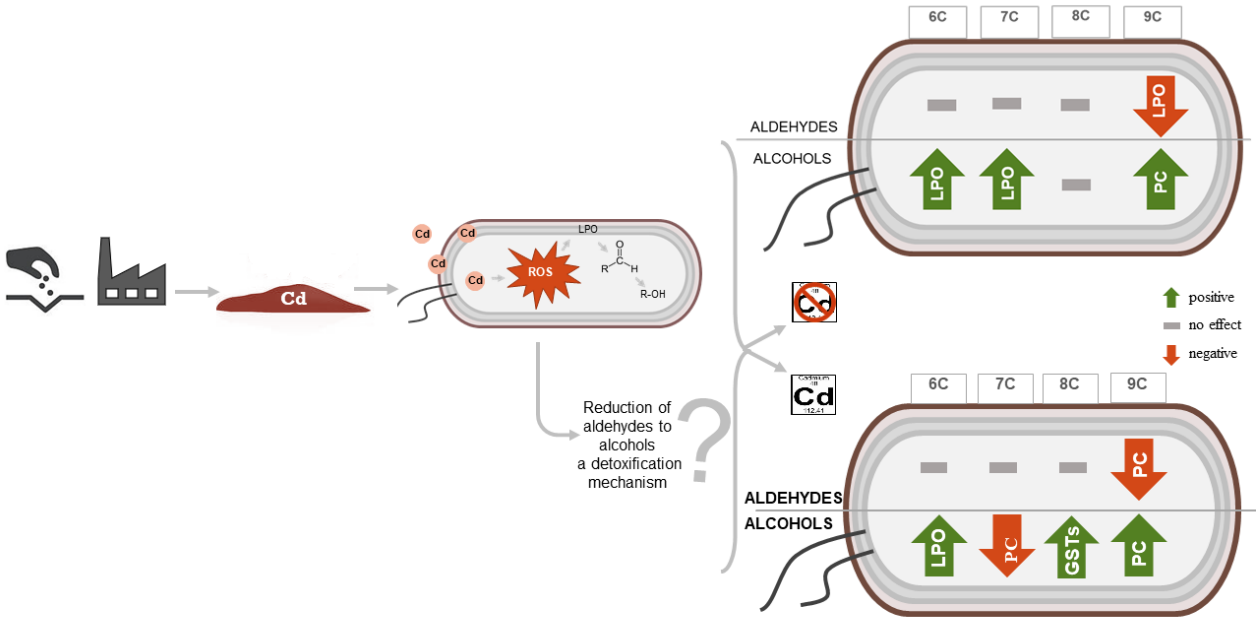
Hypothesis testing was performed by Permutation Multivariate Analysis of Variance (PERMANOVA) [36]. All analysis were performed with the software PRIMERv6 [37] with the add-on PERMANOVA+ [38]. A matrix was used to run the PERMANOVA tests, 9999 Monte Carlo permutations were considered. The pseudo-F values in the main tests were evaluated in terms of the significance and, when significant ($p \leq 0.05$) pairwise comparisons were performed between conditions. Values of $p \leq 0.05$ revealed if the groups differed significantly. The null hypotheses tested were: (a) for a given Cd level (presence or absence of Cd) and for each compound no significant differences exist among concentrations; (b) for a given concentration no significant differences exist between presence and absence of Cd; (c) for a given concentration and Cd level no significant differences exist between an aldehyde and the corresponding conjugated alcohol.

In order to analyse if the global biochemical response of *Rhizobium* was influenced by the compounds in the presence and absence of Cd, the data (fourth root transformed, normalize and the resemblance matrix normalization (Euclidean distance)) were submitted to an ordering analysis performed by Principal Coordinates (PCO), using the PRIMER 6 & PERMANOVA+. In this program it was carried out the normalization of the data.

The heatmaps representing the biochemical parameters and growth for each compound and condition were built using MetaboAnalyst 4.0 [39]. Data was normalized by weight of each replicate, and autoscaled by concentrations.

Chapter 3 - The role of volatiles in *Rhizobium* tolerance to cadmium: effects of aldehydes and alcohols on growth and biochemical endpoints

(Ecotoxicology and Environmental Safety, in press)



Abstract

Rhizobia have a significant agronomic and environmental role and are eminent contributors to soil fertility. However, this group of microorganisms are affected by various environmental stresses, such as Cd contamination. High Cd concentrations change bacterial metabolism. During this metabolic shift, bacteria alter their volatilome (the set of volatile metabolites synthesized by an organism). In the presence of Cd, peak areas of saturated aldehydes and alcohols were previously reported to increase, and the consequences of this increase to cells are poorly known. In this study, *Rhizobium* sp. strain E20-8 cells were exposed to Cd and aldehydes or their conjugated alcohols. Exposure to Cd (100 μM) inhibited cell growth and induced several biomarkers of oxidative stress. The present study also evidenced the higher toxicity of most aldehydes relatively to the corresponding alcohol in the presence of Cd, suggesting that reduction of aldehydes into alcohols

may be an effective mechanism to restrain aldehydes toxicity in *Rhizobium* cells under Cd toxicity. Nonetheless, the protective effect was dependent on the pair aldehyde-respective alcohol considered and it differed between Cd stressed and non-stressed cells. Differences in the ability to convert aldehydes to alcohols may emerge as a new feature helping explain the oxidative tolerance variability among bacteria.

3.1. Introduction

The soil is the habitat of a plethora of organisms, whose survival is dependent on the conditions surrounding them. Small changes can have a strong impact on these organisms [40]. The accumulation of toxic metals in the soil is one of the factors affecting soil organisms, including bacterial communities [1]. In agricultural soils, cadmium (Cd) concentrations have been increasing due to farming practices focused on crop yield increase [41]. The amount of available Cd in soils varies between 1.8-53 μM [5, 42, 43], however in contaminated soils the concentration can be much higher, reaching 2669 μM at highly contaminated sites [5, 44].

Cd is a very toxic element even at low concentrations [16, 45] and is classified in seventh place in the Priority List of Hazardous Substances [46]. Exposure to metals changes bacteria metabolism [47]. During this metabolic shift, bacteria alter their metabolome, including the volatilome (all the volatile metabolites synthesized by an organism) [18]. Bacterial volatile organic compounds (VOCs) are generally produced by catabolic pathways, including glycolysis, proteolysis and lipolysis, and belong to different chemical classes (e.g. terpenes, hydrocarbons, aldehydes, ketones, alcohols, acids, nitrogen containing compounds, sulphur-containing compounds) [48, 49]. Several bacterial VOCs were shown to influence growth, differentiation and stress resistance in fungi, plants and invertebrates [50–54]. However, the influence of VOCs on bacteria is scarce and the existing studies prioritize pathogenic bacteria and fungi [55, 56] or model bacteria such as *Escherichia coli* and *Bacillus subtilis* [21, 50, 57].

The volatilome of *Rhizobium* sp. strain E20-8 exposed to Cd was screened by Cardoso et al. [1] and significant increases in the relative peak areas of saturated aliphatic aldehydes and alcohols of different sizes were detected. Aldehydes have been widely described as being toxic to humans [58], most information being related to α,β-unsaturated aldehydes [22], and little information being available on saturated aldehydes. Ishino et al. [59] reported that H_2O_2 and to a lesser extent alkyl hydroperoxides are capable of mediating covalent modification of proteins by saturated aldehydes. Thus, saturated aldehydes in combination with H_2O_2 or ROOH (present at high concentrations in oxidatively stressed cells) may contribute to the modification of proteins and to cell damage under oxidative stress. As the toxicity of saturated aldehydes is poorly understood, further studies are

required to elucidate the biological consequences of production of saturated aldehydes in cells under oxidative stress [60].

Given the potentially harmful influence of aldehydes on cellular metabolism, transformation of aldehydes into less reactive or more water soluble (which can more easily be excreted) compounds can be efficient mechanisms to reduce the toxicity of aldehydes, such as the reduction of aldehydes to alcohols [27]. A gene encoding an alcohol dehydrogenase was identified in *Rhizobium* [61, 62] and aldo-keto reductases, which also reduce aldehydes to alcohols [27], are a superfamily of enzymes found in a wide variety of organisms including bacteria [63]. The presence of these enzymes in bacteria may support the increase in saturated alcohols observed in cells of *Rhizobium* exposed to Cd [1]. However, alcohols were also described to alter lipid bilayer properties, membrane function and protein function through direct interaction [28].

Despite saturated aldehydes and alcohols increase in cells exposed to oxidative stress, their toxicity is largely unknown, most of the available information is on eukaryotic cells. Since the mechanisms triggered to counteract their toxicity are poorly understood, the present study was designed to elucidate the airborne effects of both saturated aldehydes and alcohols in bacterial cells exposed or not to metal induced stress. Moreover, this study also aims to mimick one of the alterations induced by Cd in *Rhizobium* cells, and to find out if reduction of aldehydes to alcohols can be a detoxification mechanism in bacterial cells.

To achieve these goals, *Rhizobium leguminosarum* strain E20-8 cells were exposed to four saturated aldehydes and their corresponding alcohols at a wide range of concentrations (1 nM to 100 mM) in the absence (no Cd) and presence of Cd (100 μM), in a total of 12 conditions per compound. The effects of each aldehyde and the corresponding alcohol on growth, membranes (lipid peroxidation), proteins (protein content and protein carbonylation), and antioxidant and biotransformation mechanisms (superoxide dismutase, glutathione peroxidase and glutathione-S-transferases) were determined and compared.

3.2. Results

The effects of four aldehydes and four alcohols separately or in combination with Cd are shown in Figures 6, 7, 8 and 9. In each figure the effect of an aldehyde and the corresponding alcohol is compared in the presence and absence of Cd.

3.2.1. Effects of hexanal and hexanol

In cells growing without Cd, hexanal did not cause growth differences at any of the concentrations tested (Fig. 6A). Hexanol also did not change cell growth at the two lower concentrations, but from 10 μM to 100 mM growth increased between 30 and 42% relatively to

control. Cd significantly decreased cell growth. The effect of hexanal on growth was positive (but not statistically significant) compared to sole exposure to Cd at the three lowest concentrations, but this effect disappeared at higher concentrations. Hexanol was able to minimize the effects of Cd, with significantly higher growth than exposure to Cd alone (Fig. 6A and Supplementary Table S1).

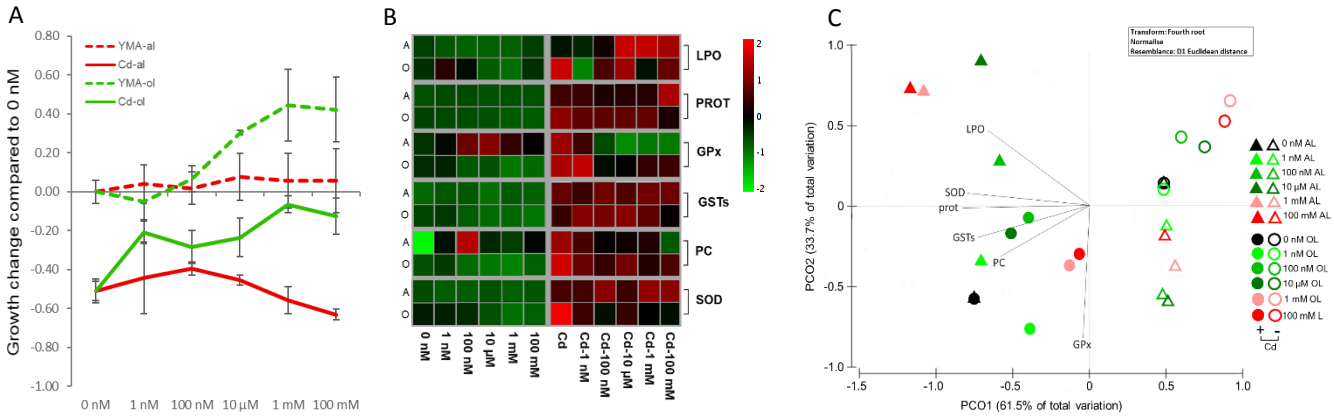


Figure 6 – Growth, antioxidant and biotransformation activity and damage in *Rhizobium* cells exposed to Cd and hexanal or hexanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM) of hexanal (A) or hexanol (O) in a total of 24 conditions. (**A**) growth variation relatively to control (no Cd no compounds). Cells were exposed to: hexanal and not to Cd (red dashed line); hexanal and Cd (red full line); hexanol and not to Cd (green dashed line); hexanol and Cd (green full line). Values are means of 3-6 replicates ± standard errors. For statistical significance see Supplementary Table S1. (**B**) Heatmap of the biochemical determinants for each condition: lipid peroxidation (LPO); protein (PROT); glutathione peroxidase (GPx); glutathione S-transferases (GSTs); protein carbonylation (PC); and superoxide dismutase (SOD). For means, standard errors and statistical significance see Supplementary Table S1. (**C**) Principal Coordinates with centroids ordination (PCO) of the biochemical determinants for each condition. Cells exposed to: hexanal and not to Cd (open triangles); hexanal and Cd (closed triangles); hexanol and not to Cd (open circles); hexanol and Cd (closed circles); detailed color scheme in the figure. Pearson correlation vectors were imposed: LPO; PC; PROT; SOD; GSTs activity and GPx activity ($r \geq 0.90$).

Heatmap analysis (Fig. 6B) evidences Cd as the major driver of biochemical change in cells, inducing the activity of antioxidant and biotransformation enzymes, such as SOD, GPx and GSTs. However, exposure to hexanal and hexanol also induced biochemical changes in either the absence or presence of Cd. In the absence of Cd hexanal did not change protein levels, GSTs and SOD activity, increased PC and GPx activity and decreased LPO. Hexanol tended to decrease the activity of the three enzymes and cell damage (only significantly in membranes (LPO). In the presence of Cd hexanal influence on enzymes activity was contradictory, not affecting GSTs, decreasing GPx and increasing SOD activity. The presence of hexanal reduced the damage caused by Cd in proteins (only significant at 100 nM), but exacerbated membrane damage (200%) at higher concentrations. Hexanol

decreased SOD and GPx activity, did not change GSTs activity, and reduced the cellular damage both in membranes (LPO) and in proteins (PC) induced by Cd (Fig. 6B and supplementary table S2).

Principal Components Ordination (PCO) analysis of the biochemical determinants for each condition tested evidenced that together PCO1 and PCO2 explained 95.2% of the total variation obtained among conditions (Fig. 6C). Along PCO1 (61.5%), two groups were clearly separated, conditions without Cd on the positive side and conditions with Cd on the negative side of the axis. PCO2, explained 33.7% of total variation. In conditions without Cd, PCO2 separated the five conditions with hexanal (open triangles) on the negative side from conditions without VOCs or with hexanol (open circles) on the positive side of the axis. In conditions with Cd, PCO2 separated the conditions without VOCs or with hexanol (closed circles) on the negative side from conditions with hexanal (closed triangles) on the positive side. From PCO analysis it was possible to observe that most biochemical markers were strongly correlated with PCO1 and more related with Cd conditions, evidencing the effort of cells to fight oxidative stress imposed by exposure to Cd. LPO was more correlated with the three higher hexanal concentrations and Cd (red, pink and dark green closed triangles), evidencing the higher damage caused by the combined exposure to Cd and the higher hexanal concentrations on membranes. On the other hand, GPx was more correlated with the combined exposure to Cd and 1 nM, 1mM and 100 mM hexanol (light green, pink and red closed circles, respectively) and with 100 nM and 10 μM hexanal (green and dark green open triangles, respectively), as evidenced by the higher GPx activity in these conditions (Fig. 6C).

3.2.2. Effects of heptanal and heptanol

In cells growing without Cd, heptanal increased growth from 100 nM to 100 mM. Heptanol increased growth 43% at 100 nM, but for higher concentrations the growth stimulation was lower (24%-31%) but still significant (Fig. 7A). In the presence of Cd, the effect of both heptanal and heptanol was negligible (Fig. 7A and supplementary table S1).

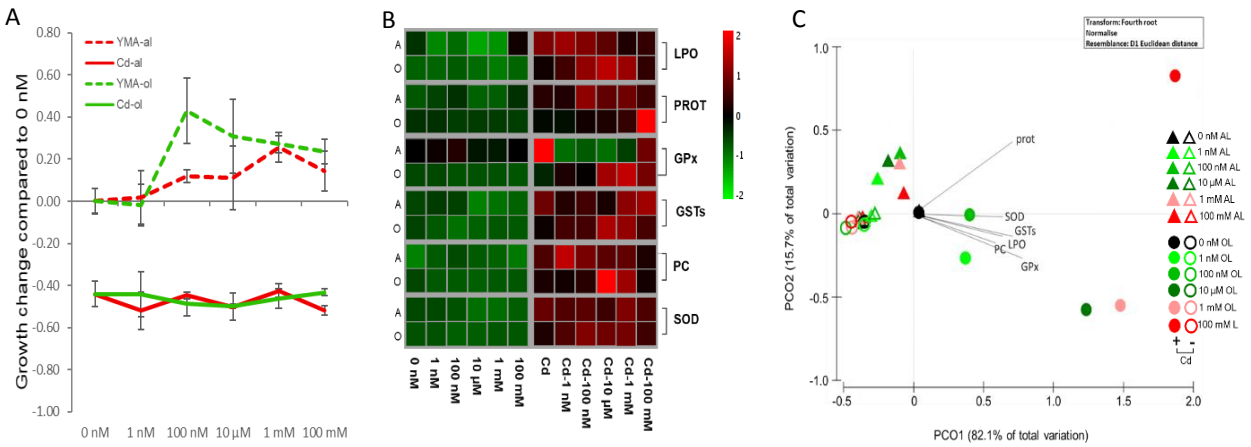


Figure 7 – Growth, antioxidant and biotransformation activity and damage in *Rhizobium* cells exposed to Cd and heptanal or heptanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM) of heptanal (A) or heptanol (O) in a total of 24 conditions. (**A**) growth variation relatively to control (no Cd no compounds). Cells were exposed to: heptanal and not to Cd (red dashed line); heptanal and Cd (red full line); heptanol and not to Cd (green dashed line); heptanol and Cd (green full line). Values are means of 3-6 replicates ± standard errors. For statistical significance see Supplementary Table S3. (**B**) Heatmap of the biochemical determinants for each condition: lipid peroxidation (LPO); protein (PROT); glutathione peroxidase (GPx); glutathione s-transferases (GSTs); protein carbonylation (PC); and superoxide dismutase (SOD). (**C**) Principal Coordinates with centroids ordination (PCO) of the biochemical determinants for each. Cells exposed to: heptanal and not to Cd (open triangles); heptanal and Cd (closed triangles); heptanol and not to Cd (open circles); heptanol and Cd (closed circles); detailed color scheme in the figure. Pearson correlation vectors were imposed: LPO; PC; PROT; SOD; GSTs activity and GPx activity ($r \geq 0.90$).

In the absence of Cd heptanal had little influence on enzymes activity, mixed influence (increases in some concentrations and decreases in others) on protein content and protein damage, and decreased membrane damage compared to control (Fig. 7B). Heptanol also had little influence on SOD and GSTs activity and on LPO levels, but decreased GPx activity, protein content and PC levels. In the presence of Cd, most parameters were not change by heptanal (but proteins increased and GPx activity decreased), whereas heptanol increased enzymes activity and cell damage at most of the concentrations, especially protein at 100 mM (Fig. 7B and supplementary table S3).

Principal Components Ordination (PCO) analysis evidenced that together PCO1 and PCO2 explained 97.8% of the total variation obtained among conditions (Fig. 5C). Along PCO1 (82.1% of total variation), three groups were clearly separated, conditions without Cd (open symbols) and conditions with Cd and heptanal (closed triangles) on the negative side, conditions only exposed to Cd (black closed triangle and circle) and to Cd and the lowest heptanol concentrations (light green and green closed circles) on the positive side but near the axis origin and conditions with Cd and highest heptanol concentrations (dark green, pink and red closed circles) on the far negative side of the axis. PCO2, explained 15.7% of total variation, and separated the five conditions with Cd and heptanal (closed triangles) and the condition with Cd and the highest heptanol concentration (red

closed circle) on the positive side from the remaining conditions (without Cd – open symbols and with Cd and the four lower heptanol concentrations – light green, green, dark green and pink closed circles) on the negative side of the axis. From PCO analysis it was possible to observe that most biochemical markers were strongly correlated with PCO1 and therefore more related with Cd and heptanol conditions. Among these, GPx was more correlated with 10 µM and 1mM heptanol concentrations and Cd (pink and dark green closed circles), evidencing GPx activity as the main mechanism to fight the joint toxicity of Cd and heptanol. On the other hand, protein was strongly correlated with Cd and 100 mM heptanol (red closed circle), evidencing the metabolic effort of cells to tolerate this condition. (Fig. 7C).

3.2.3. Effects of octanal and octanol

In cells growing without Cd, octanal did not cause significant growth differences at any of the concentrations tested although inhibitions around 10% were noticed at the lowest (1 nM and 100 nM) and highest (100 mM) concentrations and an increase of 11% was observed at 10 µM. Exposure to octanol displayed distinct influences, increases of up to 22% (10 µM) and decreases down to 20% (100 mM), that were only significant for 10 µM (Fig. 8A). In the presence of Cd, the effect of either octanal and octanol is minor (Fig. 8A and Supplementary Table S1).

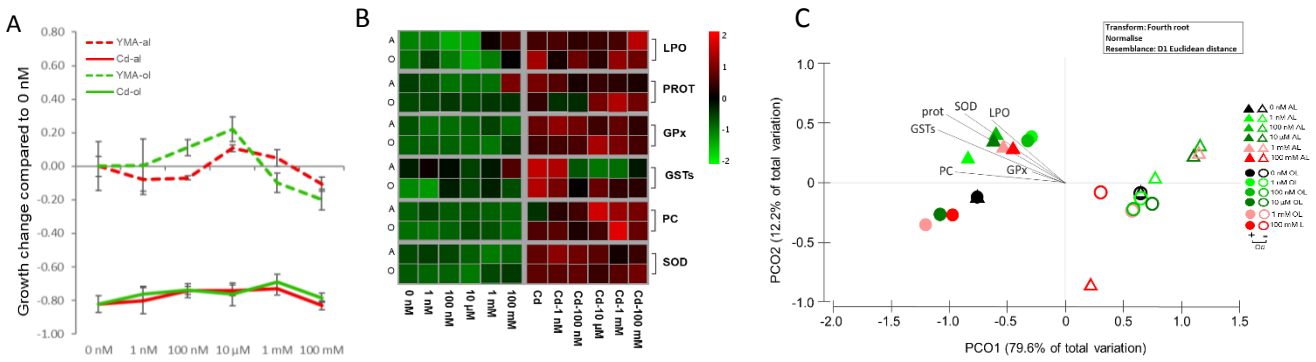


Figure 8 – Growth, antioxidant and biotransformation activity and damage in *Rhizobium* cells exposed to Cd and octanal or octanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM) of octanal (A) or octanol (O) in a total of 24 conditions. (**A**) growth variation relatively to control (no Cd no compounds). Cells were exposed to: octanal and not to Cd (red dashed line); octanal and Cd (red full line); octanol and not to Cd (green dashed line); octanol and Cd (green full line). Values are means of 3-6 replicates ± standard errors. For statistical significance see Supplementary Table S3. (**B**) Heatmap of the biochemical determinants for each condition: lipid peroxidation (LPO); protein (PROT); glutathione peroxidase (GPx); glutathione s-transferases (GSTs); protein carbonylation (PC); and superoxide dismutase (SOD). (**C**) Principal Coordinates with centroids ordination (PCO) of the biochemical determinants for each. Cells exposed to: octanal and not to Cd (open triangles); octanal and Cd (closed triangles); octanol and not to Cd (open circles); octanol and Cd (closed circles); detailed color scheme in the figure. Pearson correlation vectors were imposed: LPO; PC; PROT; SOD; GSTs activity and GPx activity ($r \geq 0.90$).

In the absence of Cd octanal did not damage cell membranes (LPO) or proteins (PC), enzymes activity was not affected either, only protein content decreased in most concentrations, yet increasing at 100 mM (Figure 8B). Octanol also had little influence on the cytoplasm, but membrane damage (LPO) increased significantly at 100 mM. In the presence of Cd, octanal did not change enzymes activity, increased protein content and damage to both membranes and proteins. Octanol did not alter the cell damage inflicted by Cd, maintained SOD and GPx activity, decreased GSTs activity, and had a dual influence on the proteins, at nanomolar range decreased and at micro and millimolar concentrations increased protein content compared to Cd alone (Fig. 8B and supplementary table S4).

Principal Components Ordination (PCO) evidenced that together PCO1 and PCO2 explained 91.8% of the total variation obtained among conditions (Fig. 8C). Along PCO1, two groups were clearly separated, conditions without Cd on the positive side and conditions with Cd on the negative side of the axis. In conditions without Cd, PCO2 separated three groups, the four lowest octanal concentrations (open triangles) on the positive side, the conditions without VOCs or with octanol (open circles) on the negative side but close to the axis origin, and the highest octanal concentration (red open triangle) on the far native side of axis 2. In conditions with Cd, PCO2 separated the conditions without VOCs or with the highest octanol concentrations (dark green, pink and red closed circles) from conditions with octanal (closed triangles) and lowest octanol concentrations (light green and green closed circles). From PCO analysis it was possible to observe that most biochemical markers were strongly correlated with PCO1 and therefore more related with Cd conditions. The highest correlations were obtained for Cd and octanal conditions, evidencing that cells exposed to the combination of Cd and octanal triggered a higher antioxidant response, but higher correlations were also obtained for LPO and PC (damage) (Fig. 8C).

3.2.4. Effects of nonanal and nonanol

In cells growing without Cd nonanal did not cause growth differences in the three lowest concentrations, but growth decreased significantly (around 25%) at highest concentrations. Nonanol did not influenced growth at 1 nM, but higher concentrations increased growth significantly (25% to 44%) (Fig. 9A). In Cd exposed cells nonanal did not change growth significantly. The five nonanol concentrations tested alleviated the negative effect of Cd on growth (28% to 43%), although this influence was not statistically significant at 100 mM (Fig. 9A and supplementary table S1).

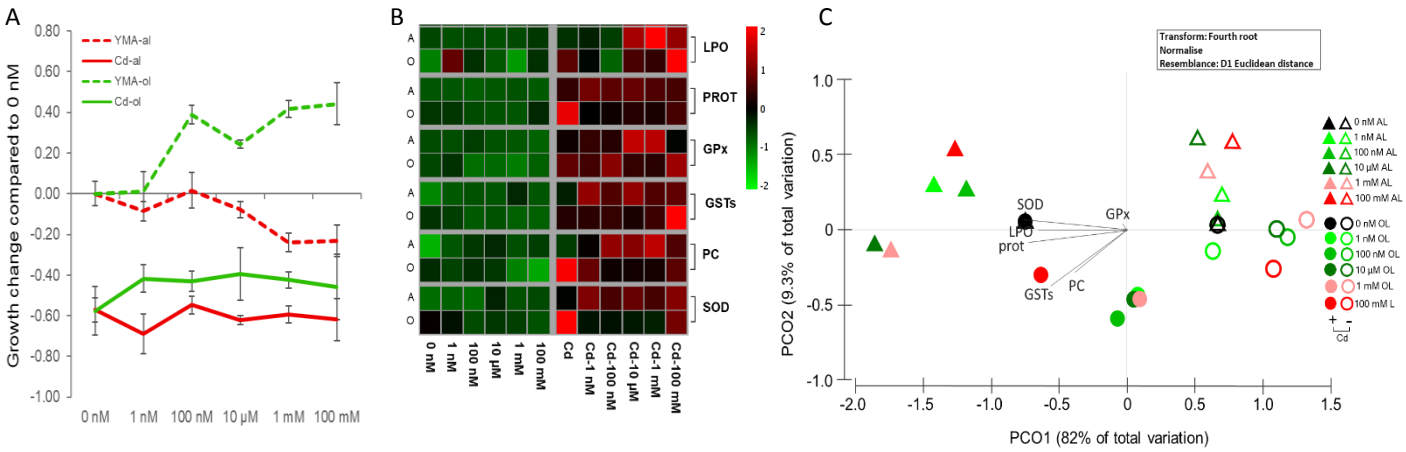


Figure 9 – Growth, antioxidant and biotransformation activity and damage in *Rhizobium* cells exposed to Cd and nonanal or nonanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM) of nonanal (A) or nonanol (O) in a total of 24 conditions. (**A**) growth variation relatively to control (no Cd no compounds). Cells were exposed to: nonanal and not to Cd (red dashed line); nonanal and Cd (red full line); nonanol and not to Cd (green dashed line); nonanol and Cd (green full line). Values are means of 3-6 replicates ± standard errors. For statistical significance see Supplementary Table S1. (**B**) Heatmap of the biochemical determinants for each condition: lipid peroxidation (LPO); protein (PROT); glutathione peroxidase (GPx); glutathione s-transferases (GSTs); protein carbonylation (PC); and superoxide dismutase (SOD). (**C**) Principal Coordinates with centroids ordination (PCO) of the biochemical determinants for each. Cells exposed to: nonanal and not to Cd (open triangles); nonanal and Cd (closed triangles); nonanol and not to Cd (open circles); nonanol and Cd (closed circles); detailed color scheme in the figure. Pearson correlation vectors were imposed: LPO; PC; PROT; SOD; GSTs activity and GPx activity ($r \geq 0.90$).

In the absence of Cd, nonanal increased SOD and GSTs activity and PC levels, had a varied influence on GPx activity (at 100 nM increased and at 100 mM decreased), decreased protein content and LPO for most concentrations (1nM to 1 mM), but at 100 mM LPO increased significantly compared to control. Nonanol decreased the activity of the three enzymes and the protein damage, did not change protein content, and most concentrations increased LPO levels compared to control. In the presence of Cd, nonanal exacerbated Cd effects in all parameters at most of the concentrations used, especially LPO at higher concentrations. Nonanol decreased SOD activity, protein and PC levels, maintained GPx and GSTs activity and LPO levels, compared to Cd alone (Fig. 9B and supplementary table S5).

Principal Components Ordination (PCO) analysis evidenced that together PCO1 and PCO2 explained 91.3% of the total variation obtained among conditions (Fig. 9C). Along PCO1, three groups were clearly separated, conditions without Cd on the positive side (open symbols), conditions with Cd and without VOCs (black closed symbols) or with Cd and nonanol (closed circles) next to

the origin or on the less negative side of the axis, and conditions with Cd and nonanal (closed triangles) on the far negative side of axis 1. PCO2, explained 9.3% of total variation. Most conditions with nonanal (triangles) with or without Cd are on the positive side of the axis 2, and most conditions with nonanol (circles) are on the negative side of axis 2. From PCO analysis it was possible to observe that most biochemical markers were strongly correlated with PCO1 and with Cd conditions, especially when nonanal was also applied, evidencing that cells exposed to the combination of Cd and nonanal triggered a higher antioxidant response, but also inflicted more damage both in membranes (LPO) and proteins (PC) (Fig. 9C).

3.3. Discussion

The results obtained in this study show that aldehydes and alcohols affect the growth and metabolism of *Rhizobium* cells on the other side of the plate, showing that these compounds were able to occupy the entire atmosphere of divided petri dishes and proving evidence that aldehydes and alcohols synthesized by a bacterial cell can influence bacterial cells at least several centimeters apart. Indeed, Repetto et al. [64] reported aldehydes as highly stable compounds that diffuse and attack targets far from the site of origin. Despite the similar chemical nature, the four aldehydes and the four alcohols studied caused different effects on *Rhizobium* cells either in the absence and presence of Cd, allowing to infer about the toxicity of these compounds and if the transformation of aldehydes to the corresponding alcohols constitute a mechanism of toxicity mitigation in cells.

3.3.1. Absence of Cd

In the absence of Cd two alcohols (octanol and nonanol) and one aldehyde (nonanal) caused membrane damage as shown by LPO values, evidencing that alcohols had higher impact in membrane stability. McKarns et al. [65] described alcohols to alter the integrity of biological membranes. Andersen and Ingo [28] reported that alcohols interact with lipid bilayers and present a chain-length-dependent lipid bilayer-alcohol interaction [28], which is in agreement with our results since hexanol decreased, heptanol did not change and octanol and nonanol increased LPO. Interference with the electron transport chain (ETC) functioning can increase the levels of reactive oxygen species (ROS) [66]. Interference may arise, among other factors, due to the destabilization of membranes [67], evidencing the potential of some aldehydes and alcohols to increase ROS. When interacting with membrane polyunsaturated fatty acids (PUFAs) ROS originate lipid peroxides whose metabolism additionally produces aldehydes and alcohols [1, 19, 20, 27], both saturated and unsaturated [22].

When aldehydes and alcohols reach the cytosol, they are free to interact with intracellular molecules such as proteins. Alcohols can interfere with proteins function through direct interactions [68] and sensitivity of proteins to alcohols was assigned to specific protein regions [28]. Saturated aldehydes tend to react with lysine residues (Lys) in proteins [22]. Since many enzymes possess Lys residues involved in the catalysis and the activity of many others is regulated by post-translational modifications of Lys residues [69] the impact of aldehydes on cell metabolism is expected to be high [59, 70–75]. Results from our study show that proteins were more susceptible to aldehydes than to alcohols. Indeed, protein carbonylation (PC) increased in cells exposed to some concentrations of the aldehydes tested. Alcohols induced less damage to proteins and in some conditions PC even decreased.

Hexanal, heptanal and octanal had little influence on cell metabolism (protein levels) and antioxidant and biotransformation activities were not triggered, yet nonanal strongly impacted cytosol, increasing protein damage, reducing protein levels and triggering SOD and GSTs activity. On the other hand, the four alcohols tested did not induce negative effects or even protected proteins (lower PC in some heptanol and nonanol concentrations), and the antioxidant and biotransformation activities (SOD, CAT and GPx) were maintained or reduced, evidencing the protective effect of these alcohols towards cytosol. However, octanol and nonanol induced membrane damage. The lower energy investment in cytosolic antioxidant activity and repair in cells exposed to alcohols, may have left more resources for growth. In fact, results show increased growth of cells exposed to alcohols relatively to control condition and to most concentrations of the corresponding aldehyde. This effect is especially evident when comparing nonanol with nonanal, with the alcohol having a positive and the aldehyde a negative effect on growth compared to control. The reduction of aldehydes to alcohols as a mechanism to reduce aldehydes toxicity was already described in eukaryotes [27] and suggested for prokaryotes [1]. The present study evidenced that reduction of aldehydes to alcohols may act as an effective mechanism to restrain aldehydes toxicity in bacterial cells such as *Rhizobium*. However, results of the present study also show that the degree of protection depends on the pair aldehyde-respective alcohol considered (Fig. 10).

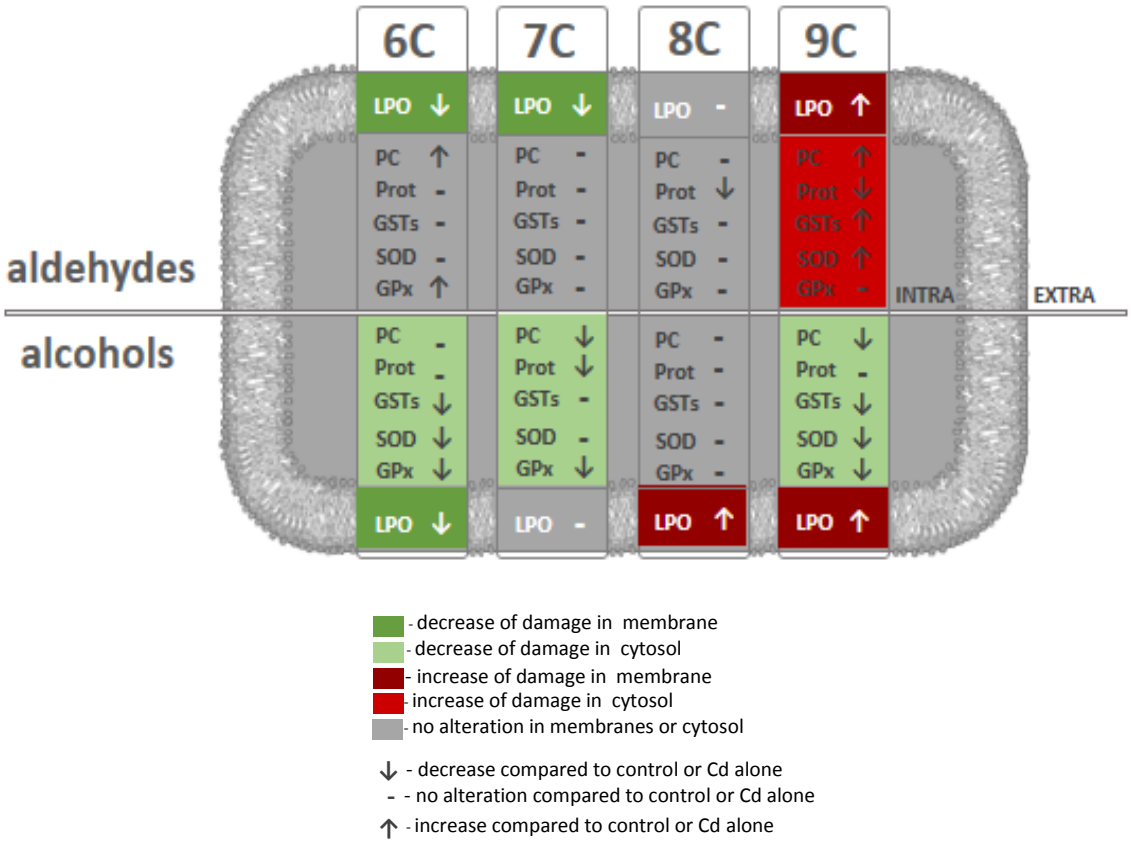


Figure 10 - Biochemical status of *Rhizobium* cells exposed to saturated aldehydes or alcohols. C6- hexanal and hexanol, C7- Heptanal and heptanol, C8- octanal and octanol, C9- Nonanal and nonanol. Variation (increase - ↑, decrease - ↓, no change - -) in biochemical parameters (lipid peroxidation - LPO, protein carbonylation – PC, protein – Prot, glutathione s-transferases – GSTs, superoxide dismutase – SOD, and glutathione peroxidase - GPx) relatively to control (non-exposed cells). Overall cell status compared to control (red – worse, green – better, gray- no change) in the cytosol and membranes.

3.3.2. Presence of Cd

It is well known that Cd is toxic to *Rhizobium* cells, causing damage to membranes, proteins and activating antioxidant and biotransformation mechanisms to combat the oxidative stress generated by Cd [5, 16, 17]. Since Cd effects on *Rhizobium* were already object of discussion in other studies [3, 5, 17, 45], the significant increase induced by Cd in all the parameters determined in the present study was not discussed. However, the effects of simultaneous exposure of Cd and aldehydes or alcohols to bacteria are virtually unknown and therefore the effects of combined exposure to these compounds and Cd were compared with sole exposure to Cd and differences thoroughly discussed in order to assess the influence of aldehydes and alcohols on Cd toxicity.

Most aldehydes exacerbated (especially at higher concentrations) the damage inflicted by Cd in membranes and proteins. Little influence of aldehydes on antioxidant and biotransformation enzymes activity (except for nonanal) was noted, possibly due to the increase of proteins. A rise in the copy number of enzymes, especially those sensitive to Cd stress, may compensate for the decrease in activity [76] that some enzymes may experience due to native conformation alterations. The alcohols hexanol, octanol and nonanol did not influence or even protected (hexanol) membranes from Cd damage. In cytosol, alcohols decreased protein damage caused by Cd, protein level and antioxidant and biotransformation activities, evidencing the protective role of alcohols in Cd exposed cells. The maintenance of GSTs activity, which convert toxic compounds resulting from peroxydized polyunsaturated fatty acids [20, 77], which interact with proteins, into less reactive compounds [78], may have protected proteins from oxidation and may explain the decrease in protein damage observed even when LPO was not reduced (octanol and nonanol). On the contrary, heptanol potentiated Cd toxicity, both in membranes and proteins. Although the activity of the three enzymes was increased, the induction of antioxidant and biotransformation mechanisms was not enough to restrain damage.

The present study also evidenced the higher toxicity of most aldehydes relatively to the corresponding alcohol in the presence of Cd, suggesting that reduction of aldehydes into alcohols may be an effective mechanism to restrain aldehydes toxicity in *Rhizobium* cells under Cd toxicity. Nonetheless, the protective effect was once again dependent on the pair aldehyde-respective alcohol considered and it differed from non-stressed cells (Fig. 11).

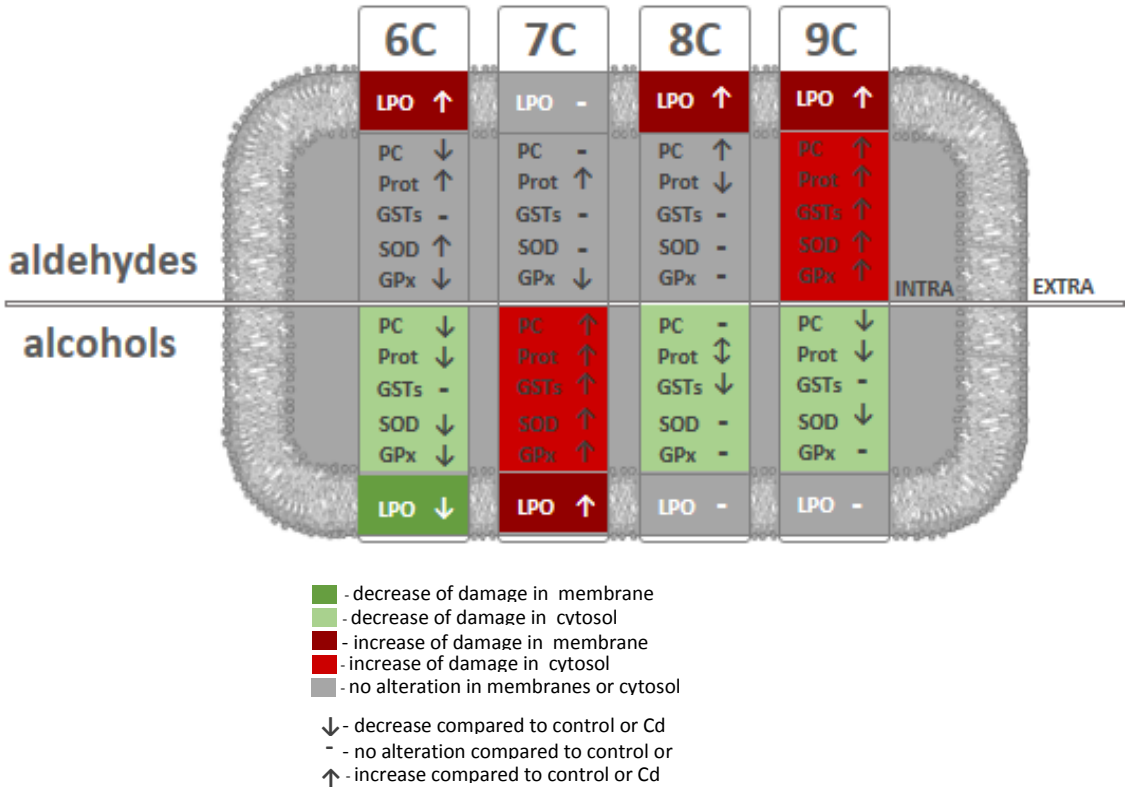
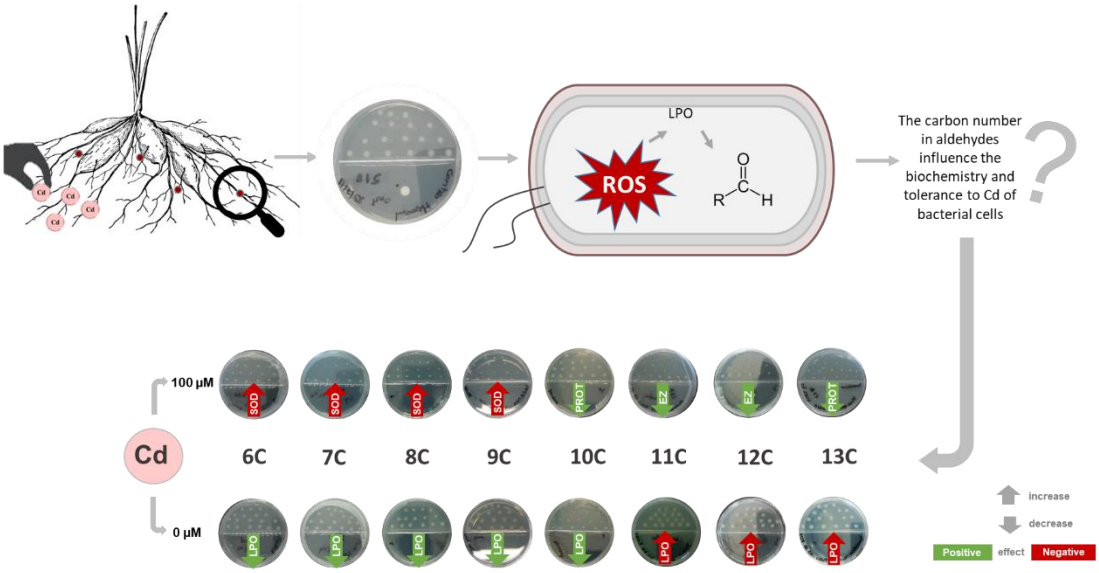


Figure 11 – Biochemical status of *Rhizobium* cells exposed to Cd and saturated aldehydes or alcohols. C6- hexanal and hexanol, C7- Heptanal and heptanol, C8- octanal and octanol, C9- Nonanal and nonanol. Variation (increase - ↑, decrease - ↓, no change - -) in biochemical parameters (lipid peroxidation - LPO, protein carbonylation – PC, protein – Prot, glutathione s-transferases – GSTs, superoxide dismutase – SOD, and glutathione peroxidase - GPx) relatively to Cd (cells only exposed to 100 µM Cd). Overall cell status compared to Cd (red – worse, green – better, gray- no change) in the cytosol and membranes.

Chapter 4 – *Rhizobium* sensing of airborne saturated aldehydes modulates the response to Cd exposure



Abstract

Saturated aldehydes are volatile compounds that can be originated from several molecules in microorganisms, namely from fatty acids, pyruvate, alcohols or aminoacids. α,β-unsaturated aldehydes are generally reported as being toxic, however for saturated aldehydes information is scarce. In a previous work, saturated aliphatic aldehydes (C6 to C13) peak areas were reported to increase in *Rhizobium* cells as a response to Cd exposure. However, the effects of these compounds on cells, challenged or not by Cd, are poorly known. Herein we report the effects on growth and biochemical endpoints related to oxidative stress of *Rhizobium* colonies under airborne exposure to hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal (C6 to C13). *Rhizobium* cells were exposed in a control condition and under Cd induced stress. Our results show that smaller aldehydes (C6 to C10) and larger aldehydes (C11 to C13) had distinct effects on some of the biochemical endpoints, for instance smaller aldehydes (C6 to C10) induced negative variations in cell LPO (-37% to -50%), most of them significant, while larger aldehydes (C11 to C13) influence resulted in positive variations, most of which between 20% and 30% that were not significant, but some significant variations (>70%) were also detected. In general, the activity of superoxide dismutase was also decreased by smaller aldehydes and increased by the larger ones. Exposure to the

aldehydes did not influence colony growth, both in control and Cd condition, although exposure to 1 mM decanal and 10 μM tridecanal led to significantly increased growth in the Cd condition. Thus, even an exposure at a distance to saturated aldehydes is able to influence the biochemical status of bacterial cells, and the effects appear to be dependent on the carbon number and thus distinct properties (e.g volatity and lipossibility) of the aldehydes. This suggests that these molecules may play unrevealed roles in the interactions in soil communities.

4.1. Introduction

Soil contamination is a global problem, affecting urban, agricultural and natural systems [79]. In agricultural soils cadmium (Cd) concentration has been gradually increasing due to farming practices to increase crop yields, such as the application of phosphate fertilizers, which may contain relatively high amounts of Cd [80]. Cd is classified in seventh place in the Priority List of Hazardous Substances (Agency for Toxic Substances and Disease Registry, 2017) and is a very toxic element, even at low concentrations [1, 13, 45, 81]. Since Cd cannot be degraded, the risk of environmental exposure is constantly increasing because of accumulation via the food [82].

Legumes are important as food, feed, and in maintaining soil fertility, which is linked to the ability of this plant family to establish an endosymbiosis with rhizobia, to fix atmospheric nitrogen and to make nitrogen available to these plants. Given the importance of legumes and the effects of toxic metals on their microsymbionts and on biological nitrogen fixation received, much attention has been given to the study of the resistance of these bacteria to toxic metals like Cd [16, 45]. Above certain levels toxic metals negatively affect rhizobia persistence in the soil, in the rhizosphere of plants, and biological N_2 fixation [45, 83, 84].

Exposure to metals induces alterations in bacterial cells with production of compounds that in turn can also affect cells. Cardoso et al. [1] reported that saturated aliphatic aldehydes (C6 to C13) were one of the groups of compounds that most increased in *Rhizobium* cells exposed to Cd. However, the effects of these compounds on cells, challenged or not by Cd, are poorly known. Aldehydes are generated from a wide variety of endogenous and exogenous precursors during numerous physiological processes, including the biotransformation of endogenous compounds such as amino acids, carbohydrates, and lipids [23, 58, 85]. More than 200 aldehyde species arise from the oxidative degradation of cellular membrane lipids [86]. Unlike free radicals, aldehydes are relatively long-lived and not only react with cellular components in the vicinity of their formation but, through diffusion or transport, also affect farther targets [86–88]. While there is a large number of references on the toxicity of α,β-unsaturated aldehydes [22, 59, 89–94], saturated aldehydes have received little attention. Ishino et al. [59] reported that H_2O_2 and alkyl hydroperoxides (present at high concentrations in oxidatively stressed cells) can mediate the covalent modification of proteins

by saturated aldehydes, through hard biological nucleophiles (e.g. primary nitrogen groups on lysine residues) [22]. Therefore, it is plausible to infer that enzymes in which lysine residues are involved in catalysis may be especially affected by higher concentrations of saturated aldehydes. Moreover, covalent post-translational modifications of proteins create an intricate layer of proteome modulation [95], also in prokaryotes [96], and lysine is one of the most heavily modified residues [95]. Protein modifications at specific Lys residues have the potential to alter the activity of the proteins [95]. If Lys residues are covalently bound to aldehydes, they cannot be post-translationally modified, reinforcing that the increase of saturated aldehydes may have a strong impact on cell metabolism. For example, hexanal, an aldehyde produced in high quantity during lipid peroxidation, showed metabolic, genotoxic and mutagenic effects, as well as inhibitory effects on proliferation of human cells [86]. However, exposure to 100 μM of saturated aldehydes (C6-C10) did not affect *Plasmodium falciparum* [97]. Hence contradictory information exists in literature, further studies are needed to clarify the effects of saturated aldehydes on cells [60].

In order to elucidate the toxicity of saturated aldehydes, identify differences in the effects caused by different aldehydes and evaluate if cell stress status influences these effects, *Rhizobium* strain E20-8 was exposed to volatile aldehydes, whose peak area was previously reported to increase in cells exposed to Cd [1]. The effect of these aldehydes was evaluated by comparing growth, antioxidant and biotransformation activities, membrane damage, and protein levels in cells challenged or not by Cd (0 and 100 μM), for six concentrations of saturated aliphatic aldehydes (C6-C13).

4.2. Results

The effect of eight saturated aliphatic aldehydes (C6 to C13) on the growth of *Rhizobium* cells in the presence and absence of Cd is represented in Figures 12 and 14 and Supplementary Table S6. The influence of aldehydes on cell biochemistry in conditions without Cd is presented as % variation compared to control (Figure 13 and Supplementary Tables S7, S8, S9, S10 and S11) and in conditions with Cd as % variation compared to sole exposure to Cd (Figure 15 and Supplementary tables S7, S8, S9, S10 and S11). Negative variations represent decreases and positive variations increases. The effects of sole Cd exposure to control condition (no Cd no compound) were already reported in several works [3, 8, 16, 17] and therefore, are not described here.

4.2.1. Absence of Cd

Growth

Most aldehydes did not have a significant influence on *Rhizobium* growth with variations relatively to control (no Cd no VOC) being less than 20% and not significant for most conditions

(Figure 12 and Supplementary Table S6). However, exposure of cells to heptanal (C7), undecanal (C11) and tridecanal (C13) led to significantly higher growth at 1 mM, 1 nM and 10 μM for C7, C11 and C13, respectively. On the other hand, exposure to octanal (C8), nonanal (C9) and dodecanal (C12) led to decreased growth, with significant variations at milimolar range for C8 and C9 and for all concentrations for C12.

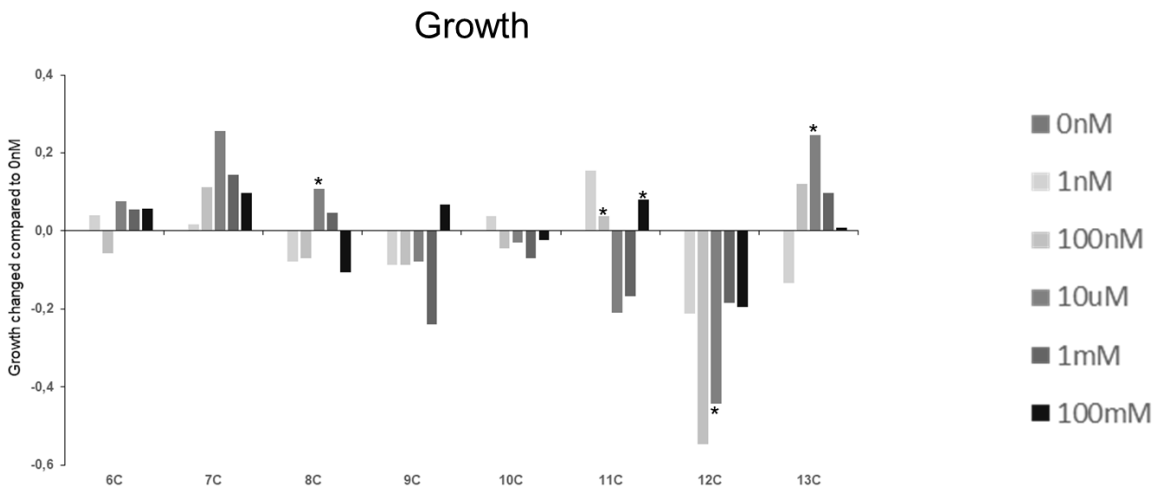


Figure. 12 - Growth of *Rhizobium* during 60 h in the absence of cadmium and in the presence of six concentrations (0nM, 1nM, 100nM, 10μ, 1mM and 100mM). of the aldehydes hexanal (6C), heptanal (7C), octanal (8C), nonanal (9C), decanal (10C), undecanal (11C), dodecanal (12C), and tridecanal (13C). Asterisks indicate significant differences between concentrations. Considered significantly different values of $p < 0.05$. For mean values, standard errors and statistical significance see Supplementary Table S6.

Biochemistry

Smaller aldehydes (C6 to C10) induced a similar response, with negative variations in cell LPO (-37% to -50%), most of them significant (Figure 13A and Supplementary Table S7). Larger aldehydes (C11 to C13) influence resulted in positive variations, most of which between 20% and 30% that were not significant; but some significant variations (>70%) were also detected. Thus, as a general trend smaller aldehydes (C6 to C10) decreased and larger ones increased lipid peroxidation.

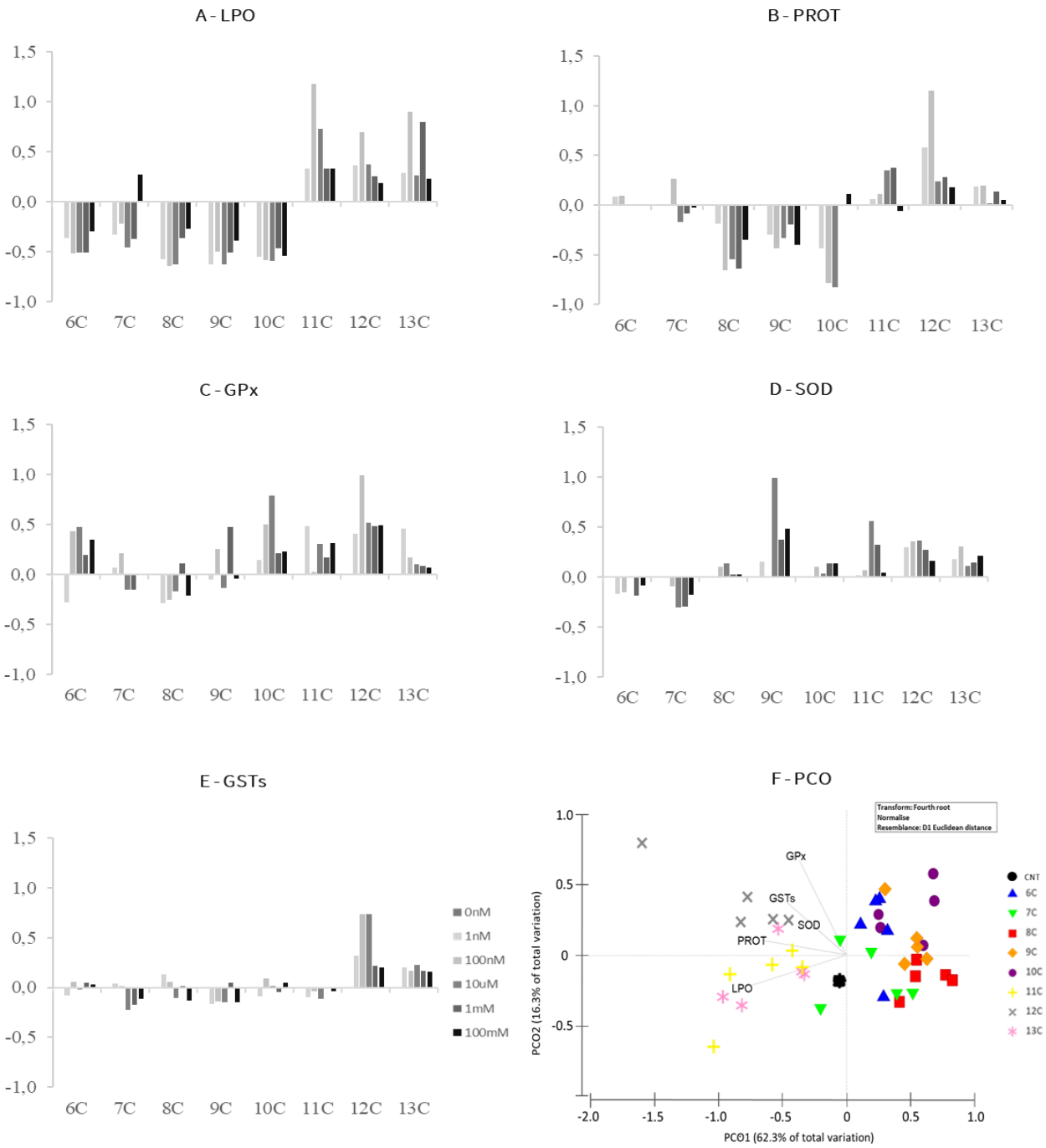


Figure. 13 – Antioxidant and biotransformation activity, damage and protein content in *Rhizobium* cells exposed to saturated aldehydes. Cells were exposed for 60h to 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM) of the aldehydes (hexanal - 6C, heptanal -7C, octanal – 8C, nonanal – 9C, decanal – 10C, undecanal – 11C, dodecanal – 12C and tridecanal – 13C). Biochemical results expressed as variation relatively to control (no Cd no compounds). **(A)** lipid peroxidation (LPO). **(B)** protein (PROT). **(C)** glutathione peroxidase (GPx). **(D)** superoxide dismutase (SOD). **(E)** glutathione s-transferases (GSTs). **(F)** Principal Coordinates Ordination of biochemical parameters in the absence (0) of cadmium and in the presence of hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal (0nM, 1nM, 100nM, 10µ, 1mM and 100mM). For mean values, standard errors and statistical significance see Supplementary Tables S7 to S11.

Hexanal (C6) and heptanal (C7) induced positive and negative variations in cell protein content, that were low (-20% to 20%) and not significant (Figure 13B and Supplementary table S7). C8 to C10 aldehydes decreased protein content, with all (C8 and C9) or most (C10) concentrations inducing negative variations (-20% to -83%), some of them significantly lower than control. Larger aldehydes (C11 to C13) increased protein content, with positive variations (2% to 116%), a few being significant. Therefore, protein content was not affected (C6 and C7) or decreased (C8 to C10) by smaller aldehydes, but was increased by larger ones.

The influence of smaller aldehydes (C6 to C9) on GPx activity is somewhat inconsistent, with some concentrations inducing negative and other positive variations, most of them not significant (Figure 13C and Supplementary table S8); among these, the effect of hexanal is more pronounced, with both significant negative (-28%) and positive (35% to 47%) variations. All concentrations of larger aldehydes (C10 to C13) increased GPx activity, some of them imposing significant variation (41 to 99%).

Smaller aldehydes (C6 and C7) reduced SOD activity, although only significantly for heptanal at 10 μM and 1 mM (Figure 13D and Supplementary table S9). Exposure to octanal (C8) had little impact (< 14%) on SOD activity. Most concentrations of larger aldehydes (C9 to C13) increased SOD activity, with few being able to induce significant variations.

Most aldehydes (C6 to C11) did not change or decreased GSTs activity (Figure 13E and Supplementary table S10). The decreasing effect was more pronounced in heptanal and nonanal with lower negative variations (down to -20%), some of which significant. The two larger aldehydes (C12 and C13) had a contrasting effect; all concentrations increased GSTs activity, with positive variations between 20% and 74%, most of them significantly different from control.

PCO analysis demonstrated differences between the bacteria exposed to smaller (C6 to C10) and larger (C11 to C13) aldehydes based on their different biochemical features (Figure 13F). GPx activity emerged as the main mechanism of distinction among larger (C12 in the positive side and C11 and C13 in the negative side of the PCO2 axis) and smaller (C10 and most C6 and C9 concentrations in the positive side and C8 and most C7 concentrations on the negative side of the axis) aldehydes.

4.2.2. Presence of Cd

Growth

The influence of aldehydes on the growth of *Rhizobium* stressed cells (exposed to Cd) was even lower than in non-stressed cells, with incipient variations relatively to sole exposure to Cd,

mostly lower than 10% and not significant (Figure 14 and Supplementary table S6). However, exposure to 1 mM decanal (C10) and 10 μM tridecanal increased significantly growth compared to sole exposure to Cd.

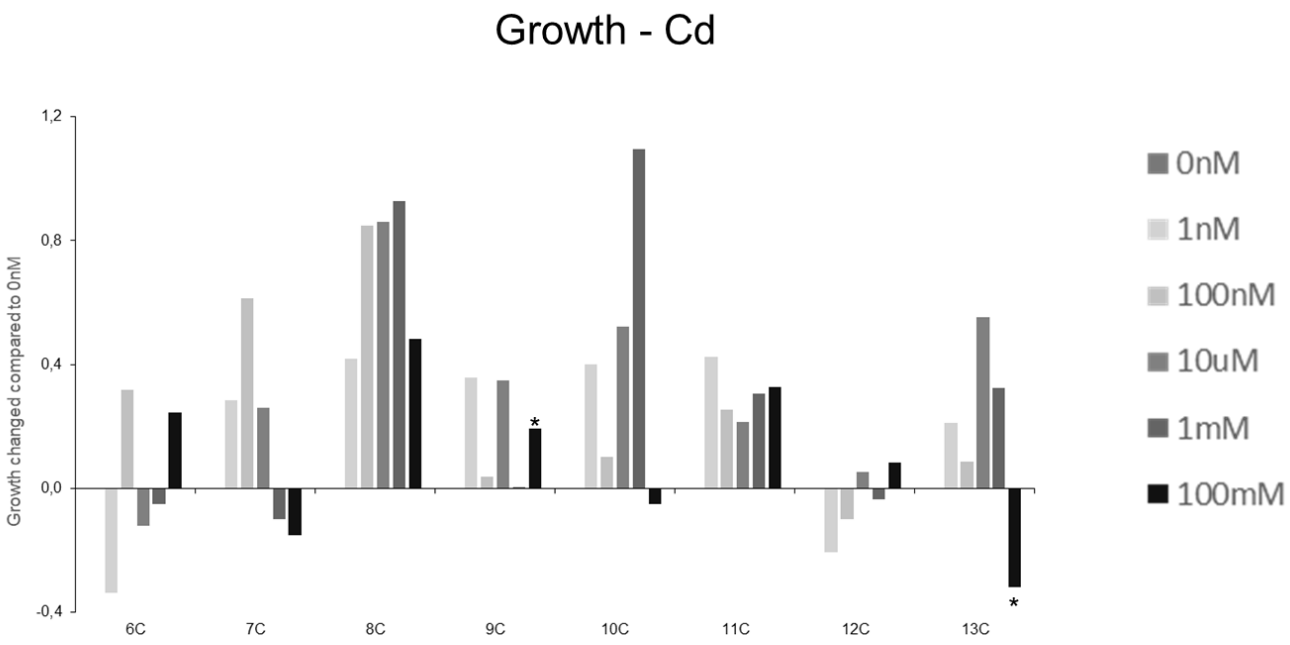


Figure. 14- Growth of *Rhizobium* during 60 h in the presence of cadmium and in the presence of six concentrations (0nM, 1nM, 100nM, 10μ, 1mM and 100mM) of the aldehydes hexanal (6C), heptanal (7C), octanal (8C), nonanal (9C), decanal (10C), undecanal (11C), dodecanal (12C), and tridecanal (13C). Asterisks indicate significant differences between concentrations. Considered significantly different values of $p < 0.05$. For mean values, standard errors and statistical significance see Supplementary Table S6.

Biochemistry

As a general trend hexanal, octanal and nonanal increased LPO compared to sole exposure to Cd, with some variations being significant (35 to 379%) (Figure 15A and Supplementary table S7).

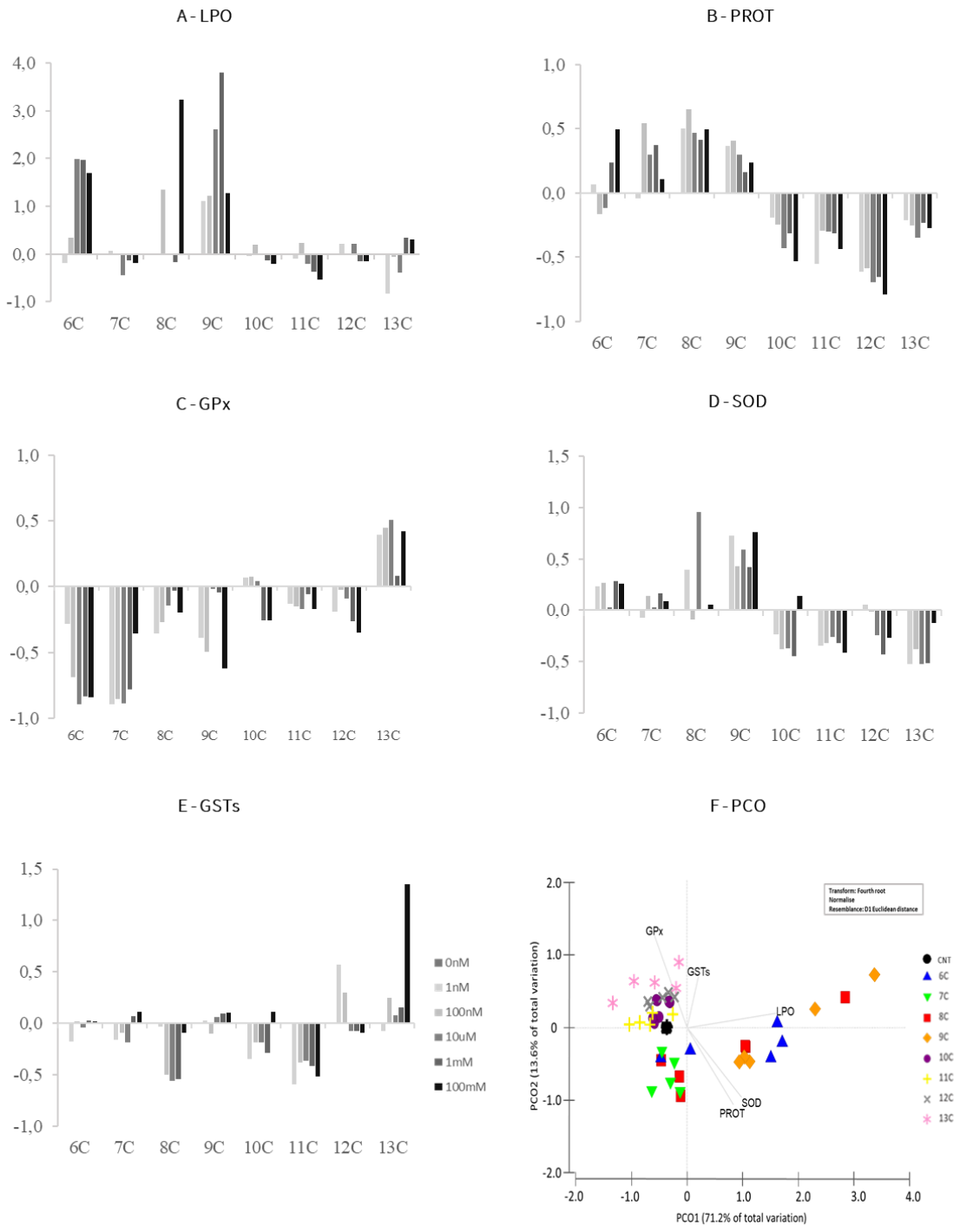


Figure. 15 – Antioxidant and biotransformation activity, damage and protein content in *Rhizobium* cells exposed to cadmium and saturated aldehydes. Cells were exposed for 60h to 100µM of Cd and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM) of the aldehydes (hexanal - 6C, heptanal -7C, octanal – 8C, nonanal – 9C, decanal – 10C, undecanal – 11C, dodecanal – 12C and tridecanal – 13C). Biochemical results expressed as variation relatively to control (no Cd no compounds). **(A)** lipid peroxidation (LPO). **(B)** protein (PROT). **(C)** glutathione peroxidase (GPx). **(D)** superoxide dismutase (SOD). **(E)** glutathione s-transferases (GSTs). **(F)** Principal Coordinates Ordination of biochemical parameters in the absence (0) of cadmium and in the presence of hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal (0nM, 1nM, 100nM, 10µ, 1mM and 100mM). For mean values, standard errors and statistical significance see Supplementary Tables S7 to S11.

Heptanal and larger aldehydes (C10 to C13) influence on LPO was small, with positive and negative variations that were only significant for exposure to 100 mM undecanal.

Smaller (C6 to C9) and larger (C10-C13) aldehydes had contrasting influence on cell protein levels (Figure 15B and Supplementary table S8). Most variations induced by smaller aldehydes are positive, with variations higher than 30% being statistically significant. All concentrations of larger aldehydes reduced protein content, with variations ranging from -19 to -70%, and most of them being statistically significant.

Aldehydes showed a contrasting influence on the activity of the three enzymes in the presence of Cd. Exposure to all concentrations of smaller aldehydes (C6 and C7) strongly (-70% to -90%) and significantly decreased GPx activity Figure 15C and Supplementary table S9). Most concentrations of C8 to C12 aldehydes decreased GPx activity, but variations were not as negative (-49% to -3%) as for smaller aldehydes and the majority were not significant. Exposure to C13 increased GPx activity; in most of the concentrations significant variations around 50% were observed.

For SOD, smaller aldehydes (C6 to C9) further increased the activity already augmented by Cd, with statistically significant variations for hexanal, octanal and especially nonanal (Figure 15D and Supplementary table S10). Distinctly, the majority of larger aldehydes (C10 to C13) concentrations significantly reduced SOD activity.

Hexanal, heptanal and nonanal had little influence on the activity of GSTs (Figure 15E and Supplementary table S11). Most concentrations of octanal and decanal and all concentrations of undecanal significantly decreased GST activity, with negative variations between -18% and -60%. Larger aldehydes (C12 and C13) showed a different trend, with some concentrations increasing significantly the activity of GSTs compared to sole exposure to Cd.

PCO analysis demonstrated differences mostly on the bacteria LPO levels; some concentrations of C6 and C8 and all concentrations of C9 with higher LPO levels on the positive side and the rest of conditions on the negative side of PCO1 (Figure 15F). The ordinate axis separated bacteria exposed to smaller (C6 to C9) from those exposed to larger (C10 to C13) aldehydes based on their different biochemical features. SOD activity and protein levels were more correlated with exposure to smaller aldehydes, while GPx and GSTs activity were more correlated with larger aldehydes.

4.3. Discussion

The levels of saturated aldehydes were shown to increase in oxidatively stressed *Rhizobium* cells [1]. The potential of these compounds to interact with biomolecules, such as proteins, led them

to be considered as toxic [22]. However, negligible effects have also been reported [97]. Airborne exposure of *Rhizobium* cells to eight saturated aldehydes, exposed or not to Cd, allowed to elucidate the effects of these compounds on bacterial cells, identify putative differences in the effects caused by each aldehyde and evaluate if cell stress status influenced these effects.

4.3.1. Absence of Cd

Due to their amphiphilic nature, aldehydes can diffuse across membranes [60]. The increase in aldehydes size increases their liposolubility, allowing higher interaction with the membrane fatty acids and temporarily destabilizing membrane integrity. Since Gram-negative bacteria present lipopolysaccharides, external and internal membranes, when passing through these lipid moieties aldehydes may temporarily destabilize membranes integrity. Smaller aldehydes present less hydrophobicity and therefore less interaction with the lipid bilayer [27]. Indeed, our study evidenced that smaller aldehydes (C6 to C10) did not increase LPO levels. On the other hand, the higher hydrophobicity of larger aldehydes can lead to higher reactivity with membrane phospholipids [98], and our study confirmed that C11 to C13 aldehydes increased membrane damage (LPO increase).
Saturated aldehydes also tend to interact with lysine residues (Lys) in proteins [22]. Due to the high number of enzymes that have catalytic Lys residues [70–75, 99] or are regulated by post-translational modifications of Lys residues [69, 100–106], the formation of adducts between saturated aldehydes and Lys residues [22] might disturb cell metabolism. In our study, soluble protein content, that represents most of the enzymatic fraction [107], was not affected (C6 and C7) or decreased (C8 to C10) by smaller aldehydes, but was increased by larger ones, evidencing the metabolic changes of cells exposed to C11, C12 and C13 aldehydes, that induced mechanisms to counteract the impact of these aldehydes. One of the mechanisms induced is the antioxidant response. SOD activity (considered the first line of defense against oxidative stress [108]) was decreased by smaller aldehydes (C6 and C7), not affected by octanal (C8) and increased by larger aldehydes (C9 to C13), evidencing the antioxidant role of the former and the pro-oxidant character of the latter. GPx activity was also induced by larger aldehydes (C10 to C13), but most concentrations of C6 also increased GPx activity. The joint action of these two enzymes reduce reactive oxygen species, such as the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) to molecules with low reactivity (H_2O and O_2) and thus with little influence on cell functioning [108, 109]. The biotransformation activity (GSTs) was not changed or was slightly reduced by most of the aldehydes tested. However, larger aldehydes (C12 and C13) increased the activity of this family of enzymes, which by conjugating glutathione with endo-xenobiotics, like lipid hydroperoxides [110], will decrease their toxic effects and may justify the slightly lower in C12 and similar in C13 LPO levels compared to C11.

The integration of the biochemical endpoints (PCO) brings out the antioxidant role of smaller aldehydes (C6 to C10) in *Rhizobium* cells, expressed by the lower membrane damage and the lower activity of antioxidant and biotransformation enzymes. Exposure to 100 μM of saturated aldehydes (C6-C10) also did not affect *Plasmodium falciparum* [97]. On the contrary, this study shows that larger aldehydes (C11 to C13) imposed higher oxidative stress that the antioxidant and biotransformation mechanisms were not able to quash and damage overcame.

4.3.2. Presence of Cd

Due to saturated aldehydes toxicity [22, 28, 64], the increased levels of aldehydes in *Rhizobium* cells [1] may contribute to the overall toxicity induced by Cd. Cd effects on *Rhizobium* were already reported [3, 5, 17, 45] and for this reason they were not object of discussion in this study. However, the contribution of aldehydes to Cd toxicity on bacteria are poorly understood and is therefore the main focus of discussion.

Hexanal (C6), octanal (C8) and nonanal (C9) exacerbated the membrane damage inflicted by Cd, but the influence of heptanal (C7) and larger aldehydes (C10 to C13) was small, not inflicting additional stress to cell membranes. Smaller (C6 to C9) and larger (C10 to C13) aldehydes also had a contrasting influence on proteins, with the former increasing and the last decreasing protein levels. An increase in protein levels evidenced the metabolic effort of cells to protect cytosolic components from the additive effects of the joint exposure to Cd and smaller aldehydes. SOD activity also reflected the higher oxidative level induced by smaller aldehydes and Cd. On the other hand, GPx and GSTs activity was little influenced or even decreased by smaller aldehydes compared to sole exposure to Cd, while larger aldehydes (C12 and C13) increased the activity of GSTs. For GPx a progressively higher activity was observed as aldehydes size increase, with activities higher than Cd for all concentrations of C13. The dual antioxidant and biotransformation role [111, 112] of these two enzymes, may have protected cells from oxidative damage. Indeed, multivariate analysis correlated larger aldehydes (C10 to C13) with GSTs and GPx activity, evidencing their protective role towards Cd toxicity and may explain the reduced effect of aldehydes on *Rhizobium* growth in the presence of Cd.

Chapter 5 - Final Remarks and future work

Anthropogenic activities such as agriculture increase soil contamination and lead to changes in soil biodiversity. Specifically, the increase in Cd affects negatively microbial communities and their ecological functions in maintaining soil fertility.

Microorganisms develop survival mechanisms, for example VOCs production, to fight and survive in stressed conditions and minimize its impact in the communities. In this dissertation we analyzed aldehydes and alcohols produced by *Rhizobium* both in the presence and absence of Cd in order to clarify the mechanisms and the changes that occur, and which tools are used to minimize the impact of stressors and to restore soil functionality.

Aldehydes may not only be a set of molecules resulting from the catabolic activity of cells, but can also play an active role in the regulation of cellular metabolism, namely in the balance of cellular redox status or in the induction of mechanisms to combat stress.

Our results show that smaller aldehydes (the smaller saturated ones) may have a beneficial effect, that switches on when cells are in oxidative stress (Cd exposed), with smaller aldehydes increasing damage and larger aldehydes inducing mechanisms of protection against oxidative stress and alleviating the damage generated by Cd. Metabolic adaptation to the new intracellular conditions generated by exposure to aldehydes appears to be successful, since the cellular damage identified was not enough to cause major changes in growth. It seems that cells, whether stressed or not, are able to adapt metabolically to the new conditions generated by exposure to aldehydes, and even when presenting damage growth is not seriously affected.

The alteration of aldehydes effects when cells were in different oxidative conditions, may constitute evidence of the distinct influence that different aldehydes may have on the regulation of cellular functions.

The results obtained also pointed that alcohols are less toxic than the corresponding aldehydes, showing the relevance that reduction of saturated aldehydes into alcohols may have in the tolerance of cells to oxidative stress inducing agents like Cd. Differences in the ability to convert aldehydes to alcohols may emerge as a new feature explaining, at least in part, tolerance variability among bacteria and possibly other taxa. Further work is needed to clarify the relevance of reduction of aldehydes to alcohols in the tolerance of organisms to oxidative stress conditions.

Increase in the activity of the enzymes (overexpression, activation) involved in the conversion of aldehydes to alcohols may be regarded as a tool to increase the tolerance of bacteria in environments impacted with metals or other pro-oxidants such as agrochemicals, pharmaceuticals, nanomaterials, nanoplastics and other emerging contaminants. To make possible the development of

this tool, more effort should be made to elucidate the traits regulating the activity of these enzymes. On the other hand, the suppression (subexpression, inhibition) of these enzymes' activity may sensitize bacteria to antimicrobials, reduce resistance to antibiotics and be regarded as a new strategy to control multi-drug resistant pathogenic bacteria.

The transformation of bigger saturated aldehydes into smaller ones and further transformation into the corresponding alcohols may be a relevant mechanism to survive Cd contamination.

This information may be useful in different contexts by increasing the resilience of bacterial communities to environmental contaminants with oxidizing effect leading to the restoration of communities in affected areas or the improvement in nutrient absorption, or by sensitizing bacteria to antimicrobial agents and increasing the efficiency of antibiotics towards multi-resistant strains of pathogenic bacteria.

Chapter 6 – References

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Annex

Annex

Chapter 2 – Supplemental files

Figure S1 – Percentage inhibition of *Rhizobium* strain E20-8 when exposed to different concentrations of Cd (0, 50, 100, 200 and 300 µM).

Chapter 3 – Supplemental files

Supplementary Table 1 – Growth in *Rhizobium* cells exposed to Cd and hexanal-hexanol, heptanal-heptanol, octanal-octanol and nonanal-nonanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

condition	Growth (mg per colony)							
	hexanal	hexanol	heptanal	heptanol	octanal	octanol	nonanal	nonanol
0 nM	3.63±0.11^{ab}*	3.63±0.08^{a}	3.63±0.08^{ab}*	3.63±0.07^{a}	3.63±0.25^{a}*	3.63±0.38^{a}*	3.63±0.68^{a}*	3.63±0.21^{a}*
1 nM	3.78±0.21^{ab}	3.43±0.17^{a}*	3.69±0.31^{ab}	3.55±0.20^{a}*	3.33±0.31^{a}*	3.65±0.42^{a}*	3.30±0.01^{a}*	3.92±0.23^{a}*
100 nM	3.41±1.17^{a}	4.32±0.55^{ab}	4.06±0.64^{a}	5.25±0.67^{ab}	3.37±0.10^{a}	2.64±0.00^{a}*	3.69±0.41^{ab}*	5.29±0.17^{b}*
10 uM	3.92±0.47^{b}	4.52±0.10^{b}*	4.00±0.09^{b}	4.79±0.77^{b}*	4.04±0.06^{a}*	4.47±0.23^{b}*	3.33±0.05^{a}*	4.86±0.05^{c}*
1 mM	3.84±0.58^{ab}	5.32±0.81^{b}*	4.60±0.21^{ab}	4.65±0.05^{b}*	3.81±0.27^{a}	2.83±0.26^{a}*	2.72±0.08^{b}*	5.55±0.07^{b}
100 mM	3.85±0.71^{a}*	5.23±0.72^{b}	4.17±0.34^{a}*	4.51±0.13^{b}	3.23±0.05^{a}*	2.88±0.16^{a}*	2.74±1.04^{b}*	5.66±0.42^{b}*
Cd	1.55±0.08^{A}*	1.55±0.02^{A}	1.55±0.15^{A}*	1.55±0.07^{A}	1.55±0.11^{A}*	1.55±0.35^{A}*	1.55±0.14^{A}*	1.55±0.31^{A}*
Cd - 1 nM	2.04±0.56^{A}	2.29±0.01^{B}*	2.17±0.19^{A}	1.40±0.10^{B}*	1.62±0.79^{A}*	1.64±0.16^{A}*	1.20±0.08^{A}*	2.20±0.10^{B}*
Cd - 100 nM	2.61±0.39^{A}	2.05±0.20^{B}	1.62±0.16^{A}	1.09±0.13^{B}	2.37±0.55^{A}	1.80±0.09^{A}*	1.76±0.40^{A}*	2.14±0.11^{B}*
Cd - 10 uM	2.00±0.03^{A}	2.20±0.26^{B}*	2.15±0.23^{A}	1.20±0.01^{B}*	2.36±0.40^{A}*	1.54±0.26^{A}*	1.35±0.03^{A}*	2.29±0.58^{B}*
Cd - 1 mM	1.38±0.08^{A}	2.73±0.03^{B}*	1.55±0.05^{A}	0.84±0.01^{B}*	2.51±0.48^{A}	2.17±0.31^{A}*	1.47±0.01^{A}*	2.17±0.06^{B}
Cd - 100 mM	1.29±0.02^{A}*	2.55±0.24^{B}	1.88±0.03^{A}*	1.47±0.19^{B}	21.71±0.02^{A}*	1.46±0.14^{A}*	1.40±0.46^{A}*	2.03±0.75^{AB}*

Supplementary Table S2 – Damage (LPO, PC), protein content (PROT) and antioxidant and biotransformation (SOD, GPX, GSTs) activity in *Rhizobium* cells exposed to Cd and hexanal or hexanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

condition	LPO (nmol g^{-1})		PC (nmol g-1)		PROT (mg g^{-1})		SOD (U g^{-1})		GSTs (mU g^{-1})		GPx (mU g^{-1})	
	hexanal	hexanol	hexanal	hexanol	hexanal	hexanol	hexanal	hexanol	hexanal	hexanol	hexanal	hexanol
0 nM	1.94±0.05^{a}	1.94±0.43^{a}	0.86±0.45^{a}	0.86±0.16^{a}*	46.46±1.77^{a}*	46.46±3.51^{a}*	62.58±12.50^{a}*	62.58±4.88^{a}*	35.05±1.12^{a}*	35.05± 3.14^{a}*	55.78± 6.43^{a}*	55.78± 5.75^{a}
1 nM	1.22±0.24^{ab}*	2.42±0.49^{a}	1.73±0.20^{b}	0.97±0.50^{a}	50.40±10.78^{a}	43.75±0.49^{a}*	51.78±4.09^{a}	61.12±0.33^{a}*	32.39±3.38^{a}	37.31± 3.95^{ab}	74.34±30.48^{ab}	58.82± 11.02^{a}*
100 nM	0.93±0.1^{b}*	2.12±0.32^{a}	2.48±0.59^{b}	0.72±0.07^{a}	50.70±5.31^{a}*	41.93±5.66^{a}*	52.76±4.21^{a}*	57.62±10.42^{a}	36.90±4.55^{a}	30.35± 4.54^{a}	126.95± 39.38^{b}	35.63± 0.73^{b}*
10 uM	0.95±0.34^{b}*	1.68±0.20^{a}*	0.78±0.43^{a}	0.66±0.07^{a}	46.00±0.46^{a}*	39.76±2.20^{a}*	62.21±5.04^{a}*	45.40± 1.13^{b}	34.24±3.80^{a}	27.67± 1.76^{b}	138.09± 24.81^{b}*	38.52± 2.97^{b}*
1 mM	0.95±0.13^{b}*	1.60±0.43^{ab}	1.56±0.34^{b}	0.60±0.15^{a}*	46.73±7.62^{a}*	34.36±7.18^{a}*	50.86±11.49^{a}*	35.07±6.16^{b}*	36.71±8.11^{a}	28.17± 4.96^{b}*	103.00± 30.08^{b}	22.54± 5.61^{c}
100 mM	1.36±0.30^{ab}*	1.26±0.15^{b}	1.78±0.37^{b}	0.79±0.13^{a}*	46.95±7.30^{a}*	34.30±5.47^{a}*	57.10±12.16^{a}*	40.87±7.71^{b}*	36.09±7.83^{a}	25.70± 3.62^{b}*	82.87± 0.49^{ab}*	27.88± 3.12^{c}*
Cd	3.32±0.57^{A}	3.32±0.99^{A}	2.37±0.31^{A}	2.37±0.10^{A}*	149.43±11.22^{A}*	149.46±0.44^{AB}*	149.94±9.67^{A}*	149.94±12.66^{A}*	58.44±7.42^{A}*	58.44± 7.88^{A}*	148.97± 10.47^{A}*	148.97± 62.46^{A}
Cd -1 nM	2.66±0.17^{A}*	1.47±0.24^{B}	2.06±0.32^{A}	1.84±0.34^{B}	126.38±34.30^{A}	127.76±31.08^{A}*	146.51±41.41^{AB}	90.76±7.78^{B}*	48,16±5,63^{A}	73.38± 4.87^{A}	106.93± 91.78^{AB}	159.26± 7.14^{A}*
Cd -100 nM	4.47±1.00^{A}*	2.61±0.47^{Ac}	1.35±0.22^{B}	1.58±0.32^{B}	124.68±1.77^{A}*	127.05±22.14^{AB}*	133.93±3.52^{A}*	86.79±14.22^{B}	59,64±10,54^{A}	71.14± 4.72^{A}	46.16± 4.70^{B}	68.88± 1.45^{B}*
Cd -10 uM	9.94±0.28^{B}*	3.06±0.25^{A}*	1.86±0.05^{A}	1.26±0.65^{B}	131.91±6.02^{A}*	129.16±25.00^{AB}*	142.09±13.54^{A}*	88.83±20.61^{B}	55,98±8,12^{A}	73.52± 15.10^{A}	15.78± 1.85^{C}*	75.62± 5.18^{B}*
Cd -1 mM	9.87±0.80^{B}*	2.10±0.17^{c}	1.92±0.48^{A}	1.47±0.10^{B}*	184.26±35.79^{A}*	99.05±9.17^{B}*	193.04±46.55^{B}*	70.84±6.99^{Bc}*	60,13±10,76^{A}	66.05± 0.78^{A}*	24.85± 3.57^{BC}	98.08± 46.75^{B}
Cd-100 mM	8.97±0.22^{B}*	2.75±0.66^{Ac}	1.82±0.13^{A}	1.69±0.10^{B}*	223.40±38.78^{A}*	111.25±17.68^{AB}*	189.19±12.29^{B}*	66.86±2.32^{c}*	59,63±5,10^{A}	49.31± 5.08^{A}*	24.20± 5.18^{BC}*	99.63± 5.31^{B}*

Supplementary Table S3 – – Damage (LPO, PC), protein content (PROT) and antioxidant and biotransformation (SOD, GPX, GSTs) activity in *Rhizobium* cells exposed to Cd and heptanal or heptanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

condition	LPO (nmol g^{-1})		PC (nmol g^{-1})		PROT (mg g^{-1})		SOD (U g^{-1})		GSTs (mU g^{-1})		GPx (mU g^{-1})	
	heptanal	heptanol	heptanal	heptanol	heptanal	heptanol	heptanal	heptanol	heptanal	heptanol	heptanal	heptanol
0 nM	1.94 ± 0.12^{a}	1.94±0.02^{a}*	0.86±0.12^{a}*	0.86±0.12^{a}	46.46± 3.18^{a}*	46.46± 10.99^{a}*	62.58±10.38^{a}*	62.58±2.50^{a}*	35.05±0.70^{a}	35.05±2.70^{a}*	55.78±9.35^{a}*	55.78±13.25^{a}*
1 nM	1.30 ± 0.09^{b}	1.88 ± 0.26^{a}	1.15±0.15^{a}	0.56±0.09^{b}*	63.58±17.71^{b}*	43.83± 14.00^{a}*	63.20±8.52^{a}*	62.96±5.46^{a}*	36.28±4.50^{a}	28.91±2.63^{ab}*	59.71±9.02^{a}*	59.96±13.11^{a}*
100 nM	1.51± 0.18^{ab}	2.00± 0.15^{a}*	1.46±0.09^{b}*	0.52±0.03^{b}	77.29± 9.35^{b}*	12.23± 0.84^{b}*	56.64±7.65^{a}	53.39±2.46^{a}*	35.66±5.71^{a}	21.61±1.13^{b}*	67.85±15.70^{a}*	26.83±10.01^{b}*
10 uM	1.04±0.01^{c}*	1.81± 0.20^{a}*	1.14±0.12^{a}	0.44±0.09^{b}*	38.36± 2.80^{a}*	15.48± 0.61^{b}*	43.25±2.52^{a}*	55.33±11.35^{a}*	27.25±2.43^{a}	40.07±2.22^{a}	47.41±4.38^{a}*	33.77±0.56^{b}*
1 mM	1.20± 0.15 ^{bc}*	1.59± 0.14^{a}*	0.68±0.05^{c}*	0.56±0.07^{b}*	42.46± 4.30^{a}*	18.01± 2.01^{b}*	43.98±5.27^{a}*	59.46±5.50^{a}*	29.01±2.75^{a}	29.55±1.17^{ab}*	47.06±4.74^{a}	33.26±4.02^{b}*
100 mM	2.46 ± 0.38^{a}	1.89± 0.23^{a}*	0.93±0.10^{a}*	0.57±0.20^{b}*	45.09± 1.70^{a}*	27.31± 5.73^{c}*	51.60±3.04^{a}*	59.22±14.90^{a}*	31.11±1.40^{a}	28.85±5.70^{ab}	55.71±5.90^{a}*	23.87±5.12^{b}*
Cd	3.32 ± 2.31^{A}	3.32± 0.17^{A}*	2.37±0.34^{A}*	2.37±0.70^{A}	149.46± 2.70^{A}	149.46±19.93^{A}*	149.94±24.18^{A}*	149.94±18.08^{A}*	58.44±0.13^{A}	58.44±7.79^{A}*	148.97±2.26^{A}*	148.97±11.98^{A}*
Cd-1 nM	3.55 ± 1.28^{A}	3.91± 0.71^{AB}	3.22±1.38^{A}	2.24±0.12^{A}*	142.74±17.91^{A}*	137.05± 11.36^{A}*	139.08±16.01^{A}*	187.96±22.81^{B}*	49.02±9.73^{A}	74.41±11.19^{B}*	15.68±7.07^{B}*	318.22±83.0^{B}*
Cd-100 nM	3.34 ± 1.00^{A}	4.92± 0.60^{B}*	3.48±0.07^{A}*	2.59±1.00^{A}	230.93±36.15^{B}*	227.73± 46.10^{B}*	143.79±30.75^{A}	226.41±37.31^{B}*	52.94±6.16^{A}	74.69±6.84^{B}*	22.11±2.01^{B}*	259.25±36.04^{B}*
Cd-10 uM	3.04 ± 0.19^{A}	5.41± 0.59^{B}*	2.48±0.59^{A}	5.99±1.21^{B}*	194.17± 5.14^{B}*	249.87± 26.61^{B}*	129.36±21.70^{A}*	212.72±26.15^{B}	47.38±7.27^{A}	104.48±10.18^{c}	17.12±5.14^{B}*	666.47±25.92^{c}*
Cd-1 mM	2.62±0.19^{A}*	5.01± 0.27^{B}*	2.97±0.18^{A}*	4.44±1.14^{AB}*	205.52± 3.51^{B}*	314.90± 44.05^{c}*	162.56±17.40^{A}*	217.65±13.87^{B}*	62.21±8.92^{A}	96.97±8.99^{c}	32.22±11.28^{B}	751.63±237.91^{c}
Cd-100 mM	2.71±0.22^{A}*	3.73±0.47^{AB}*	2.02±0.05^{A}*	2.28±0.23^{A}*	165.47±10.45^{A}*	109.28±118.54^{D}*	137.27±9.96^{A}*	175.39±14.09^{AB}*	64.86±2.92^{A}	75.17±4.11^{B}	96.17±7.31^{c}*	547.34±207.22^{Bc}*

Supplementary Table S4 – Damage (LPO, PC), protein content (PROT) and antioxidant and biotransformation (SOD, GPX, GSTs) activity in *Rhizobium* cells exposed to Cd and octanal or octanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

condition	LPO (nmol g^{-1})		PC (nmol g^{-1})		PROT (mg g-1)		SOD (U g^{-1})		GSTs (mU g^{-1})		GPx (mU g^{-1})	
	octanal	octanol	octanal	octanol	octanal	octanol	octanal	octanol	octanal	octanol	octanal	octanol
0 nM	1.94±0.05^{a}	1.94±0.18^{a}	0.86±0.00^{a}	0.86±0.10^{a}	46.46± 7.02^{a}*	46.46±4.40^{a}	62.58±3.47^{a}*	62.58±12.83^{ab}*	35.05±5.00^{a}	35.05±4.09^{ac}*	55.78±21.43^{a}*	55.78±4.95^{a}*
1 nM	1.94±0.21^{a}	2.21±0.45^{ab}	1.82±0.48^{a}	0.84±0.12^{a}	37.57± 9.15^{a}*	50.05±0.08^{a}	62.58±11.55^{a}*	57.34±8.47^{ab}*	39.56±5.71^{a}*	33.81±4.31^{a}*	39.61±4.18^{a}	57.08±16.04^{a}*
100 nM	1.95±0.27^{a}	1.87±0.08^{a}	1.09±0.16^{a}*	0.95±0.14^{a}*	16.06± 1.07^{b}*	56.97±7.47^{a}	69.16±7.38^{a}*	57.76±13.73^{a}*	36.93±1.37^{a}*	43.07±1.68^{b}*	41.52±9.88^{a}*	53.75±3.07^{a}*
10 uM	1.95±0.37^{a}*	1.45±0.17^{a}	1.17±0.21^{a}*	1.14±0.49^{a}*	21.16± 6.50^{b}*	47.80±0.45^{a}	71.32±14.35^{a}	47.67±4.54^{a}*	31.28±0.91^{a}*	26.05±2.22^{c}*	46.34±5.56^{a}	50.20±6.77^{a}*
1 mM	1.66±0.11^{a}*	1.86±0.20^{a}	0.88±0.08^{a}*	1.09±0.15^{a}*	16.61± 0.48^{b}*	59.40±6.56^{a}	63.98±3.02^{a}	71.61±12.68^{ab}*	35.48±4.36^{a}*	42.86±5.99^{a}*	61.94±12.74^{a}*	42.07±4.88^{a}
100 mM	1.86±0.07^{a}*	2.51±0.03^{b}	0.84±0.02^{a}*	0.90±0.05^{a}*	150.37± 9.14^{c}*	62.42±15.24^{a}	46.49±7.57^{a}*	77.48±9.73^{a}*	46.46±26.20^{a}*	39.44±4.29^{a}*	43.71±14.66^{a}	79.45±15.63^{a}*
Cd	2.94±0.43^{A}*	3.32±1.03^{A}	2.37±1.04^{A}	2.37±1.86^{A}	149.46± 34.71^{A}	149.46±43.95^{A}	122.10±17.78^{AB}*	149.94±21.65^{A}*	58.44±10.08^{A}	58.44±4.47^{A}*	148.97±20.80^{A}*	148.97±14.64^{A}*
Cd-1 nM	3.24±0.48^{A}	2.61±0.13^{A}	5.38±1.22^{B}	2.45±0.53^{A}	224.15± 121.74^{A}	63.66±11.89^{B}	145.13±3.71^{A}*	146.51±24.88^{A}*	56.51±26.67^{A}*	53.91±14.17^{AB}*	168.61±73.15^{A}	151.29±7.59^{A}*
Cd-100 nM	5.06±1.08^{B}	3.02±1.20^{A}	6.18±0.98^{B}*	1.97±0.25^{A}*	247.02± 87.00^{A}	70.12±11.54^{B}	156.38±18.14^{A}*	150.58±15.85^{A}*	29.27±8.09^{A}*	45.44±5.72^{A}*	153.10±32.96^{A}*	154.63±8.91^{A}*
Cd - 10 uM	3.34±0.16^{A}*	2.70±0.79^{A}	10.87±0.29^{c}*	2.27±0.25^{A}*	219.19± 130.66^{A}	207.94±98.41^{Ac}	80.74±17.27^{B}	131.95±19.91^{A}*	25.93±3.46^{A}*	52.78±1.42^{A}*	127.74±37.09^{A}	210.32±47.66^{A}*
Cd-1 mM	5.81±1.12^{B}*	3.23±0.82^{A}	9.15±0.32^{c}*	3.53±0.52^{B}*	210.83± 126.08^{A}	257.42±7.01^{c}	110.72±17.27^{AB}	145.54±19.92^{A}*	26.92±6.00^{A}*	48.86±3.65^{A}*	144.58±19.49^{A}*	182.62±68.12^{A}
Cd-100 mM	4.12±0.47^{B}*	3.06±0.70^{A}	7.97±0.61^{Bc}*	2.56±0.11^{A}*	223.24± 146.14^{A}	202.31±107.77^{Ac}	129.08±11.62^{AB}*	159.03±12.79^{A}*	35.71±2.16^{A}*	48.24±4.75^{A}*	119.34±41.06^{A}	152.40±21.09^{A}*

Supplementary Table S5 – – Damage (LPO, PC), protein content (PROT) and antioxidant and biotransformation (SOD, GPX, GSTs) activity in *Rhizobium* cells exposed to Cd and nonanal or nonanol. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

condition	LPO (nmol g^{-1})		PC (nmol g^{-1})		PROT (mg g^{-1})		SOD (U g^{-1})		GSTs (mU g^{-1})		GPx (mU g^{-1})	
	nonanal	nonanol	nonanal	nonanol	nonanal	nonanol	nonanal	nonanol	nonanal	nonanol	nonanal	nonanol
0 nM	1.94 ± 0.28^{a}	1.94 ± 0.32^{a}	0.86±0.14^{a}	0.86±0.06^{a}	46.46±16.66^{a}*	46.46±5.30^{a}*	62.58±11.99^{a}*	62.58±7.85^{a}*	35.05±3.71^{a}*	35.05±4.66^{a}*	55.78±.,72^{a}	55.78±12.13^{a}*
1 nM	1.23 ± 0.21^{a}*	3.39 ± 0.97^{b}	2.03±0.02^{b}	0.73±0.08^{a}*	32.43±1.80^{ab}*	44.99±2.22^{a}*	71.98±.,69^{a}*	54.10±1.35^{a}	45.13±3.12^{b}	32.56±3.69^{a}*	53.01±11.96^{a}	67.38±7.08^{a}
100 nM	1.63 ± 0.41^{a}	2.45 ± 0.57^{b}	2.42±0.15^{b}	0.84±0.03^{a}	26.34±3.07^{b}	37.07±0.84^{a}*	61.87±8.83^{a}*	33.91±2.32^{b}*	46.57±6.30^{b}	24.71±1.28^{b}*	70.16±8.95^{b}*	32.55±5.83^{b}*
10 uM	1.21 ± 0.30^{a}*	2.20 ± 0.23^{ab}	2.59±0.21^{b}	0.80±0.28^{a}	30.94±1.12^{b}	34.73±1.09^{a}*	124.52±6.10^{b}	41.12±0.66^{b}	46.04±2.07^{b}*	26.96±1.96^{b}*	48.43±5.75^{a}	36.19±4.39^{b}*
1 mM	1.62 ± 0.26^{a}*	1.77 ± 0.19^{a}	2.1±0.04^{b}	0.40±0.03^{b}*	37.24±1.79^{ab}*	33.60±0.55^{a}*	86.09±6.71^{c}*	38.22±7.06^{b}*	56.56±4.57^{b}	23.52±0.48^{b}*	44.24±2.31^{a}*	25.07±5.15^{b}*
100 mM	2.78 ± 0.54^{b}*	2.45 ± 0.25^{b}	2.35±0.13^{b}	0.22±0.02^{c}*	27.73±7.39^{b}*	49.22±5.76^{a}	92.64±14.09^{c}*	31.92±0.84^{b}*	46.13±13.46^{b}	23.82±2.07^{b}*	30.97±0.53^{c}*	41.13±5.16^{ab}*
Cd	3.32 ± 0.75^{A}	3.32 ± 0.36^{A}	2.37±0.82^{A}	2.37±0.60^{A}	149.46±4.24^{A}*	149.46±15.78^{A}*	149.94±2.87^{A}*	149.94±9.74^{A}*	58.44±1.19^{A}*	58.44±2.57^{A}*	148.97±38.84^{A}	148.97±24.91^{A}*
Cd - 1 nM	4.14 ± 0.44^{A}*	2.72 ± 1.00^{A}	3.20±0.55^{AB}	1.61±0.19^{B}*	204.13±22.39^{B}*	64.24±6.28^{B}*	258.63±44.99^{B}*	53.12±5.47^{B}	99.13±18.65^{B}	59.92±7.44^{A}*	189.47±71.02^{AB}	131.95±26.94^{A}
Cd - 100 nM	2.35 ± 0.44^{A}	2.12 ± 0.14^{A}	4.98±0.74^{B}	1.16±0.19^{B}	138.64±51.81^{A}	69.98±9.71^{B}*	214.29±8.87^{B}*	54.23±3.24^{B}*	83.99±51.85^{B}	62.85±7.68^{A}*	174.37±34.06^{AB}*	172.13±27.96^{A}
Cd - 10 uM	16.85 ± 3.63^{B}*	3.22 ± 0.70^{A}	4.81±0.65^{B}	1.19±0.18^{B}	193.92±15.20^{B}*	78.64±17.92^{B}	238.81±46.02^{B}	52.13±7.96^{B}	99.27±37.62^{B}*	56.09±8.97^{A}*	337.52±148.63^{B}*	124.41±13.48^{B}*
Cd - 1 mM	22.38 ± 1.69^{B}*	3.09 ± 0.63^{A}	5.50±1.07^{B}	1.19±0.25^{B}*	173.66±13.92^{AB}*	74.65±6.33^{B}*	212.55±8.82^{B}*	48.51±4.18^{B}	84.18±3.93^{B}	64.61±7.94^{A}*	327.82±15.67^{B}*	118.16±13.59^{B}*
Cd - 100 mM	15.65 ± 2.03^{B}*	4.40 ± 1.51^{A}	4.12±0.44^{B}	1.54±0.31^{B}*	184.29±57.18^{AB}*	90.43±26.06^{B}	264.34±15.85^{B}*	101.42±4.40^{AB}*	87.66±23.98^{B}	116.32±32.27^{A}*	130.05±25.54^{A}*	182.50±49.33^{A}*

Chapter 4 – Supplemental files

Supplementary Table S6 – Growth in *Rhizobium* cells exposed to Cd and hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

Growth	Hexanal	Heptanal	Octanal	Nonanal	Decanal	Undecanal	Dodecanal	Tridecanal
Control	$3.63\pm0.12^{*A}$	$3.63\pm0.09^{*A}$	3.63 ± 0.17^{AB}	$3.63\pm0.52^{*A}$	$3.63\pm0.06^{*A}$	$3.63\pm0.01^{*AB}$	$3.63\pm0.04^{*A}$	3.63 ± 0.14^{AB}
1nM	3.78 ± 0.14^{A}	3.69 ± 0.22^{A}	$3.33\pm0.02^{*AB}$	$3.30\pm0.10^{*A}$	$3.77\pm0.02^{*A}$	$4.21\pm0.14^{*B}$	$2.82\pm0.10^{*A}$	$3.12\pm0.06^{*A}$
100nM	3.41 ± 0.01^{A}	4.06 ± 0.11^{A}	$3.37\pm0.30^{*A}$	4.04 ± 0.07^{A}	$3.46\pm0.30^{*A}$	$3.78\pm0.04^{*AB}$	1.55 ± 0.07^{B}	$4.08\pm0.23^{*AB}$
10µM	$3.92\pm0.35^{*A}$	$4.60\pm0.08^{*A}$	$4.04\pm0.08^{*B}$	$3.33\pm0.04^{*A}$	$3.52\pm0.08^{*A}$	2.83 ± 0.05^{A}	$1.95\pm0.04^{*B}$	$4.56\pm0.11^{*B}$
1mM	$3.84\pm0.44^{*A}$	$4.17\pm0.19^{*A}$	$3.81\pm0.04^{*AB}$	$2.72\pm0.17^{*A}$	$3.37\pm0.04^{*A}$	3.00 ± 0.13^{AB}	$2.93\pm0.17^{*A}$	3.99 ± 0.77^{AB}
100mM	3.85 ± 0.55^{A}	4.00 ± 0.01^{A}	$3.23\pm0.13^{*A}$	3.89 ± 0.17^{A}	$3.54\pm0.13^{*A}$	$3.93\pm0.06^{*B}$	2.89 ± 0.17^{A}	$3.66\pm0.05^{*A}$
Cd	$1.55\pm0.03^{*A}$	$1.55\pm0.06^{*A}$	1.55 ± 0.01^{A}	1.55 ± 0.06^{AB}	$1.55\pm0.01^{*A}$	$1.55\pm0.33^{*A}$	$1.55\pm0.06^{*A}$	1.55 ± 0.05^{AB}
Cd 1nM	2.04 ± 0.42^{A}	2.17 ± 0.61^{A}	$2.27\pm0.02^{*A}$	$0.97\pm0.01^{*AB}$	$2.24\pm0.02^{*A}$	$2.28\pm0.23^{*A}$	$1.20\pm0.01^{*A}$	$1.92\pm0.05^{*A}$
Cd 100nM	2.61 ± 0.29^{A}	1.62 ± 0.42^{A}	$3.01\pm0.00^{*A}$	2.10 ± 0.09^{AB}	$1.73\pm0.00^{*A}$	$1.99\pm0.10^{*A}$	$1.38\pm0.09^{*A}$	$1.70\pm0.12^{*A}$
Cd 10µM	$2.00\pm0.05^{*A}$	$2.15\pm0.29^{*A}$	$3.03\pm0.29^{*A}$	$1.35\pm0.08^{*AB}$	$2.45\pm0.29^{*A}$	1.92 ± 0.03^{A}	1.64 ± 0.08^{A}	$2.50\pm0.05^{*A}$
Cd 1mM	$1.38\pm0.03^{*A}$	$1.55\pm0.36^{*A}$	$3.15\pm0.51^{*A}$	$1.47\pm0.02^{*A}$	$3.44\pm0.51^{*A}$	2.08 ± 0.07^{A}	1.49 ± 0.02^{A}	2.11 ± 0.05^{AB}
Cd 100mM	1.29 ± 0.04^{A}	1.88 ± 0.04^{A}	$2.38\pm0.08^{*A}$	1.97 ± 0.03^{B}	$1.46\pm0.08^{*A}$	$2.12\pm0.07^{*A}$	$1.69\pm0.03^{*A}$	$1.00\pm0.10^{*B}$

Supplementary Table S7 – Lipid peroxidation (LPO) in *Rhizobium* cells exposed to Cd and hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

LPO	Hexanal	Heptanal	Octanal	Nonanal	Decanal	Undecanal	Dodecanal	Tridecanal
Control	1.94 ± 0.05^{A}	1.94 ± 0.12^{A}	1.94 ± 0.05^{A}	1.94 ± 0.28^{A}	1.94 ± 0.11^{A}	1.94 ± 0.13^{A}	1.94 ± 0.33^{A}	1.94 ± 0.29^{A}
1nM	1.22 ± 0.24^{A}	1.30 ± 0.09^{B}	1.94 ± 0.2^{A}	1.23 ± 0.21^{A}	1.94 ± 0.23^{A}	2.58 ± 0.20^{A}	1.23 ± 0.41^{A}	1.25 ± 0.13^{A}
100nM	0.93 ± 0.1^{B}	1.51 ± 0.18^{A}	1.95 ± 0.27^{A}	1.63 ± 0.41^{A}	0.87 ± 0.08^{B}	4.22 ± 0.16^{B}	1.63 ± 0.48^{A}	6.62 ± 2.21^{B}
10µM	0.95 ± 0.34^{A}	1.04 ± 0.01^{B}	1.95 ± 0.37^{A}	1.21 ± 0.30^{A}	1.85 ± 0.61^{B}	3.35 ± 0.70^{A}	1.21 ± 0.11^{A}	0.61 ± 0.22^{A}
1mM	0.95 ± 0.13^{B}	1.20 ± 0.15^{B}	1.66 ± 0.11^{A}	1.62 ± 0.26^{A}	1.91 ± 0.59^{A}	2.59 ± 0.77^{A}	1.62 ± 0.68^{A}	6.98 ± 0.44^{B}
100mM	1.36 ± 0.30^{A}	2.46 ± 0.38^{A}	1.86 ± 0.07^{A}	2.78 ± 0.54^{A}	2.31 ± 0.40^{B}	2.58 ± 0.39^{A}	2.78 ± 0.35^{A}	0.60 ± 0.06^{A}
Cd	3.32 ± 0.57^{A}	3.32 ± 2.31^{A}	3.32 ± 0.43^{A}	3.32 ± 0.75^{A}	3.32 ± 0.21^{A}	3.32 ± 0.32^{A}	3.32 ± 1.03^{A}	3.32 ± 0.53^{A}
Cd 1nM	2.66 ± 0.17^{A}	3.55 ± 1.28^{A}	3.24 ± 0.48^{A}	4.14 ± 0.44^{A}	2.68 ± 0.71^{A}	0.97 ± 0.21^{A}	4.14 ± 1.67^{A}	0.87 ± 0.11^{A}
Cd 100nM	4.47 ± 1.00^{A}	3.34 ± 1.00^{A}	5.06 ± 1.08^{A}	2.35 ± 0.44^{A}	1.77 ± 0.48^{A}	1.09 ± 0.29^{A}	2.35 ± 1.15^{A}	4.60 ± 0.12^{A}
Cd 10µM	9.94 ± 0.28^{B}	3.04 ± 0.19^{A}	3.34 ± 0.16^{A}	16.85 ± 3.63^{B}	1.76 ± 0.21^{A}	0.85 ± 0.19^{A}	16.85 ± 0.66^{B}	2.98 ± 0.47^{A}
Cd 1mM	9.87 ± 0.80^{B}	2.62 ± 0.19^{A}	5.81 ± 1.12^{A}	22.38 ± 1.69^{B}	1.51 ± 0.37^{A}	0.67 ± 0.20^{A}	22.38 ± 0.59^{B}	6.52 ± 1.74^{A}
Cd 100mM	8.97 ± 0.22^{B}	2.71 ± 0.22^{A}	4.12 ± 0.47^{A}	15.65 ± 2.03^{B}	2.65 ± 0.04^{A}	0.52 ± 0.10^{A}	15.55 ± 0.60^{B}	6.37 ± 0.48^{A}

Supplementary Table S8 – Protein content in *Rhizobium* cells exposed to Cd and hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

PROT	Hexanal	Heptanal	Octanal	Nonanal	Decanal	Undecanal	Dodecanal	Tridecanal
Control	46.46±1.77^{*A}	46.46± 3.18^{*A}	46.46± 7.02^{*A}	46.46±16.66^{A}	46.46±14.56^{*ABC}	46.46±0.61^{*A}	46.46±1.89^{*A}	46.46±9.34^{*A}
1nM	50.40±10.78^{*A}	63.58± 17.71^{*A}	37.57± 9.15^{AB}	71.98±3.69^{A}	50.84±3.21^{*ABC}	63.36±0.99^{*A}	81.00±9.68^{*B}	49.37±5.06^{A}
100nM	50.70±5.31^{A}	77.29± 9.35^{*B}	16.06± 1.07^{B}	61.87±8.83^{A}	68.79±10.01^{BC}	66.96±5.16^{AB}	132.27±10.74^{C}	60.00±8.00^{A}
10µM	46.00±0.46^{*A}	38.36± 2.80^{*A}	21.16± 6.50^{AB}	124.52±6.10^{A}	5961±7.33^{*C}	97.72±14.36^{*B}	85.64±36.51^{AB}	46.44±4.71^{*A}
1mM	46.73±7.62^{*A}	42.46± 4.30^{*A}	16.61± 0.48^{B}	86.09±6.71^{A}	70.80±6.49^{*A}	82.50±13.93^{A}	79.80±9.31^{AB}	62.71±16.57^{*A}
100mM	46.95±7.30^{*A}	45.09± 1.70^{*A}	150.37± 9.14^{*A}	92.64±14.09^{A}	71.17±7.22^{AB}	55.49±5.64^{*A}	72.79±8.59^{AB}	50.90±2.46^{*A}
Cd	149.43±11.22^{*AC}	149.46± 2.70^{*A}	149.46±22.23^{*A}	149.46±4.24^{A}	149.46±84.50^{*A}	149.46±40.10^{*A}	149.46±60.41^{*A}	149.46±40.74^{*A}
Cd 1nM	126.38±34.30^{*AC}	142.74± 17.91^{*A}	127.86±11.50^{A}	204.13±22.39^{A}	177.83±89.06^{*A}	67.38±2.99^{*B}	57.53±10.45^{*A}	145.98±22.98^{A}
Cd 100nM	124.68±1.77^{B}	230.93± 36.15^{*B}	95.25± 9.57^{A}	138.64±51.81^{A}	112.87±16.47^{B}	105.64±19.04^{B}	61.63±17.02^{A}	184.97±24.08^{A}
Cd 10µM	131.91±6.02^{*AB}	194.17± 5.14^{*B}	111.37±16.05^{A}	193.92±15.20^{A}	85.60±1.46^{*B}	104.51±9.48^{*B}	45.55±3.9^{B}	103.19±30.85^{*A}
Cd 1mM	184.26±35.79^{*ABC}	205.52± 3.51^{*B}	105.86±12.68^{A}	173.66±13.92^{A}	131.22±23.0^{*B}	102.37±16.83^{B}	51.20±5.16^{B}	141.80±17.12^{*A}
Cd 100mM	223.40±38.78^{*C}	165.47± 10.45^{*AB}	96.37± 5.48^{*A}	184.29±57.18^{A}	70.37±14.08^{*B}	83.90±5.06^{*B}	31.10±1.9^{B}	269.45±9.62^{*A}

Supplementary Table S9 - Antioxidant activity (GPx) in *Rhizobium* cells exposed to Cd and hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

GPx	Hexanal	Heptanal	Octanal	Nonanal	Decanal	Undecanal	Dodecanal	Tridecanal
Control	55.78± 6.43^{*A}	55.78±9.35^{*A}	55.78±21.43^{*A}	55.78±7.72^{*A}	55.78±6.98^{*A}	55.78±14.40^{*A}	55.58±4.58^{*A}	55.78±1.12^{*A}
1nM	74.34±30.48^{*B}	59.71±9.02^{*A}	39.61±4.18^{*A}	53.01±11.96^{*A}	63.65±2.73^{*A}	82.73±5.98^{*A}	78.41±0.07^{*B}	81.53±1.26^{*B}
100nM	126.95±39.38^{*B}	67.85±15.70^{*A}	41.52±9.88^{*A}	70.16±8.95^{*A}	83.69±28.60^{*A}	57.37±3.89^{*A}	110.75±27.49^{*B}	65.39±11.23^{*A}
10µM	138.09 24.81^{*B}	47.41±4.38^{*A}	46.34±5.56^{*A}	48.43±5.75^{*A}	99.77±20.08^{*A}	72.86±10.81^{*A}	84.58±6.63^{*B}	61.69±4.00^{*A}
1mM	103.00±30.08^{*B}	47.06±4.74^{A}	61.94±12.74^{*A}	44.24±2.31^{*A}	67.48±15.15^{*A}	65.45±6.38^{*A}	82.47±21.48^{*B}	40.47±6.39^{*A}
100mM	82.87± 0.49^{*B}	55.71±5.90^{*A}	43.71±14.66^{*A}	30.97±0.53^{*A}	68.55±18.51^{*A}	73.42±17.84^{*A}	82.82±30.59^{*B}	59.40±7.99^{*A}
Cd	148.97±10.47^{*A}	148.97±2.26^{*A}	148.97±20.80^{*A}	148.97±38.84^{*A}	148.97±2.60^{*A}	148.97±23.46^{*A}	148.97±26.99^{*A}	148.97±41.56^{*A}
Cd 1nM	106.93± 1.78^{*A}	15.68±7.07^{*B}	168.61±73.15^{*A}	189.47±71.02^{*A}	159.49±3.76^{*A}	129.08±30.36^{*A}	82.34±9.55^{*A}	113.38±42.95^{*A}
Cd 100nM	46.16± 4.70^{*B}	22.11±2.01^{*B}	153.10±32.96^{*A}	174.37±34.06^{*A}	159.99±27.52^{*A}	126.57±13.30^{*A}	259.68±5.39^{*B}	215.11±3.12^{*B}
Cd 10µM	15.78± 1.85^{*B}	17.12±5.14^{*B}	127.74±37.09^{*A}	337.52±148.63^{*B}	168.93±15.75^{*A}	123.56±25.96^{*A}	146.48±76.69^{*A}	224.32±27.84^{*A}
Cd 1mM	24.85± 3.57^{*B}	32.22±11.28^{B}	144.58±19.49^{*A}	327.82±15.67$^{* B}$	149.89±40.07^{*A}	140.73±17.36^{*A}	90.35±10.00^{*A}	144.12±0.44^{*A}
Cd 100mM	24.20± 5.18^{*B}	96.17±7.31^{*A}	119.34±41.06^{*A}	130.05±25.54^{*A}	186.48±78.10^{*A}	123.61±9.10^{*A}	54.03±25.17^{*B}	211.54±1.30^{*B}

Supplementary Table S10 – Antioxidant activity (SOD) in *Rhizobium* cells exposed to Cd and hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

SOD	Hexanal	Heptanal	Octanal	Nonanal	Decanal	Undecanal	Dodecanal	Tridecanal
Control	62.58± 12.50^{*A}	62.58±10.38^{*A}	62.58±3.47^{*A}	62.58±11.99^{*A}	62.58±4.39^{*A}	62.58±1.73^{*A}	62.58±3.02^{*A}	62.58±15.74^{A}
1nM	51.78±4.09^{*A}	63.20±8.52^{*A}	62.58±11.55^{*A}	71.98±3.69^{*A}	50.84±3.21*^{A}	63.36±0.99^{*A}	81.00±9.68^{*A}	49.37±5.06^{A}
100nM	52.76± 4.21^{*A}	56.64±7.65^{*A}	69.16±7.38^{*A}	61.87±8.83^{*A}	68.79±10.01^{A}	66.96±5.16^{A}	132.27±10.74^{A}	60.00±8.00^{A}
10µM	62.21± 5.04^{*A}	43.25±2.52^{*A}	71.32±14.35^{*A}	124.52±6.10^{B}	59.61±7.33*^{A}	97.72±14.36^{A}	85.64±36.51^{A}	46.44±4.71^{A}
1mM	50.86± 11.49^{*A}	43.98±5.27^{*A}	63.98±3.02^{*A}	86.09±6.71^{*A}	70.80±6.49^{A}	82.50±13.93^{A}	79.80±9.31^{A}	62.71±16.57^{A}
100mM	57.10± 12.16^{*A}	51.60±3.04^{*A}	46.49±7.57^{*A}	92.64±14.09^{*AB}	71.17±7.22*^{A}	55.49±5.64^{A}	72.79±8.59^{A}	50.90±2.46^{A}
Cd	149.94± 9.67^{*AB}	149.94±24.18^{*A}	149.94±17.78^{*AC}	149.94±2.87^{*A}	149.94±12.56*^{AC}	149.94±0.26^{*A}	149.94±14.53^{*A}	149.94±10.96^{AB}
Cd 1nM	146.51± 41.41^{*A}	139.08±16.01^{*A}	145.13±3.71^{*BC}	258.63±44.99^{*B}	115.36±9.95*^{A}	60.25±3.26^{*A}	158.44±21.57^{*A}	160.06±17.90^{AB}
Cd 100nM	133.93±3.52^{*AB}	143.79±30.75^{*A}	156.38±18.14^{*AB}	214.29±8.87^{*B}	93.64±5.20^{A}	82.90±10.41^{A}	148.04±40.59^{AB}	194.41±8.94^{A}
Cd 10µM	142.09±13.54^{*B}	129.36±21.70^{*A}	80.74±1727^{*C}	238.81±46.02^{B}	95.00±1.32*^{B}	89.75±2.11^{A}	113.70±9.18^{AB}	142.61±20.23^{A}
Cd 1mM	193.04±46.55^{*AB}	162.56±17.40^{*A}	110.72±17.27^{*ABC}	212.55±8.82^{*B}	55.04±1.91^{B}	82.65±10.72^{A}	85.85±6.96^{B}	163.46±12.20^{A}
Cd 100mM	189.19±12.29^{*AB}	137.27±9.96^{*A}	129.08±11.62^{*ABC}	264.34±15.85^{*B}	170.91±30.69*^{C}	71.17±3.83^{A}	109.81±15.10^{AB}	273.59±7.04^{B}

Supplementary Table S11 – Biotransformation enzymes activity (GSTs) in *Rhizobium* cells exposed to Cd and hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal and tridecanal. Cells were simultaneously exposed to 2 Cd conditions (0 and 100 µM) and 6 concentrations (0nM, 1nM, 100nM, 10µM, 1mM and 100mM). Values are means of at least 3 replicates ± standard error; different lowercase letters indicate significant differences among compounds concentrations in no Cd (0 µM) condition; uppercase letters indicate significant differences among compounds concentrations in Cd condition, and asterisks indicate significant differences between conditions (0 and 100 µM Cd) for the same concentration of the same compound. Considered significantly different values of $p < 0.05$.

GSTs	Hexanal	Heptanal	Octanal	Nonanal	Decanal	Undecanal	Dodecanal	Tridecanal
Control	35.05± 1.12^{*A}	35.05±0.70^{*A}	35.05±5.00^{A}	35.05±3.71^{A}	35.05±0.90^{*A}	35.05±1.04^{*A}	35.05±0.71*^{A}	35.05±3.99*^{A}
1nM	32.39± 3.38^{A}	36.28±4.50^{A}	39.56±5.71^{A}	45.13±3.12^{*A}	32.06±1.48^{*A}	31.57±1.3^{*A}	46.13±2.71*^{B}	37.18±5.19*^{A}
100nM	36.90± 4.55^{A}	35.66±5.71^{A}	36.93±1.37^{A}	46.57±6.30^{A}	38.05±5.86^{A}	33.76±2.06*^{A}	60.88±9.13^{B}	36.91±6.57*^{A}
10µM	34.24± 3.80^{A}	27.25±2.43^{A}	31.28±0.91^{A}	46.04±2.07^{*A}	35.63±2.96^{*A}	30.92±4.53^{A}	60.90±5.32^{B}	42.85±2.99*^{A}
1mM	36.71± 8.11^{A}	29.01±2.75^{*A}	35.48±4.36^{A}	56.56±4.57^{*A}	33.50±2.29^{A}	35.36±4.55^{A}	42.65±6.09^{A}	33.11±5.79*^{A}
100mM	36.09± 7.83^{A}	31.11±1.40^{*A}	46.46±26.20^{A}	46.13±13.46^{A}	36.68±4.07^{*A}	33.78±1.31^{A}	42.09±5.62^{A}	40.52±2.65*^{A}
Cd	58.44± 7.42^{*A}	58.44±0.13^{*A}	58.44±10.08^{A}	58.44±1.19^{A}	58.44±8.93^{*A}	58.44±17.57^{*A}	58.44±5.78*^{A}	58.44±5.51*^{A}
Cd 1nM	48.16± 5.63^{A}	49.02±9.73^{A}	56.51±26.67^{AB}	99.13±18.65^{*A}	38.13±3.24^{*B}	23.65±1.48^{*A}	70.24±15.17*^{A}	53.98±2.38*^{A}
Cd 100nM	59.64± 10.54^{A}	52.94±6.16^{A}	29.27±8.09^{AB}	83.99±51.85^{A}	58.06±10.40^{AB}	36.04±5.65^{*A}	60.53±11.50^{A}	85.61±11.58*^{A}
Cd 10µM	55.98± 8.12^{A}	47.38±7.27^{A}	25.93±3.46^{B}	99.27±37.62^{*A}	47.77±1.61^{*B}	37.04±4.65^{A}	50.29±8.76^{A}	63.01±13.90*^{A}
Cd 1mM	60.13± 10.76^{A}	62.21±8.92^{*A}	26.92±6.00^{AB}	84.18±3.93^{*A}	41.51±3.70^{B}	34.18±5.89^{A}	50.10±2.21^{A}	67.41±9.43*^{A}
Cd100mM	59.63± 5.10^{A}	64.86±2.92^{*A}	35.71±2.16^{AB}	87.66±23.98^{A}	64.98±4.45^{*A}	28.33±1.07^{A}	49.44±4.05^{A}	137.58±7.00*^{A}