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Review

Antioxidant responses of Annelids, Brassicaceae and Fabaceae to pollutants: A review

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ABSTRACT

Pollutants, such as Metal Trace Elements (MTEs) and organic compounds (polycyclic aromatic hydrocarbons, pesticides), can impact DNA structure of living organisms and thus generate damage. For instance, cadmium is a well-known genotoxic and mechanisms explaining its clastogenicity are mainly indirect: inhibition of DNA repair mechanisms and/or induction of Reactive Oxygen Species (ROS). Animal or vegetal cells use antioxidant defense systems to protect themselves against ROS produced during oxidative stress. Because tolerance of organisms depends, at least partially, on their ability to cope with ROS, the mechanisms of production and management of ROS were investigated a lot in Ecotoxicology as markers of biotic and abiotic stress. This was mainly done through the measurement of enzyme activities The present Review focuses on 3 test species living in close contact with soil that are often used in soil ecotoxicology: the worm *Eisenia fetida*, and two plant species, *Trifolium repens* (white clover) and *Brassica oleracea* (cabbage). *E. fetida* is a soil-dwelling organism commonly used for biomonitoring. *T. repens* is a symbiotic plant species which forms root nodule with soil bacteria, while *B. oleracea* is a non-symbiotic plant. In literature, some oxidative stress enzyme activities have already been measured in those species but such analyses do not allow distinction between individual enzyme involvements in oxidative stress. Gene expression studies would allow this distinction at the transcriptomic level.

A literature review and a data search in molecular database were carried out on the basis of keywords in Scopus, in PubMed and in Genbank™ for each species. Molecular data regarding *E. fetida* were already available in databases, but a lack of data regarding oxidative stress related genes was observed for *T. repens* and *B. oleracea*. By exploiting the conservation observed between species and using molecular biology techniques, we partially cloned missing candidates involved in oxidative stress and in metal detoxification in *E. fetida*, *T. repens* and *B. oleracea*.

Contents

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1. Introduction

"Reactive Oxygen Species" (ROS) are reactive molecules produced in living cells during normal vital processes (e.g. electron transport processes in photosynthesis and aerobic respiration, enzyme-catalyzed reactions, etc.) and are generated in most cell compartments (Del Río et al., 2006; Mittler et al., 2004; Navrot et al., 2007). ROS consist in both free radicals ($O_2^{\bullet-}$, superoxide anion radical; OH●, hydroxyl radical; HO_2●, perhydroxy radical and RO●, alkoxy radicals) and non-radical (molecular) forms (H_2O_2, hydrogen peroxide and $^1O_2$, singlet oxygen) (Halliwell, 1987). Under normal conditions, there is a balance between prooxidant and antioxidant molecules where the latter provide a good protection against ROS. However, interactions between ROS and antioxidant molecules may occur. Indeed, in stress conditions e.g. – water stress (Pan et al., 2006; Wang et al., 2008), – temperature stress (Suzuki and Mittler, 2006; Lushchak, 2011), – exposure to UV radiation (Agarwal, 2007; Yang et al., 2008), – exposure to inorganic or organic contaminants (Skórzyńska-Polit et al., 2004; Mobin and Khan, 2007; Dixon et al., 2010) and – challenge by pathogens (Bolwell and Wojtaszek, 1997; Dixon et al., 2010), a disturbance in the prooxidant–antioxidant balance in favor of prooxidants may lead to potential damage (Sies, 1991). In these stressful conditions, ROS can have toxic effects on various components in cells (Kappus, 1987). For instance, it has been observed that ROS affect membrane properties, cause oxidative damage to nucleic acids, lipids and proteins making these molecules non-functional (Foyer and Noctor, 2005; Gill and Tuteja, 2010).

Animal or vegetal cells use antioxidant defense systems to get protection against ROS produced during oxidative stress. These defense systems which are distributed in all cell compartments (cytoplasm and organelles) are both non-enzymatic (e.g. glutathione, proline, α-tocopherol, carotenoids and flavonoids) or enzymatic (e.g. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), glutathione reductase (GR), etc.) (Mittler et al., 2004; Gill and Tuteja, 2010). Antioxidants maintain also ROS homeostasis in cells and allow functional intracellular machinery. ROS were first observed during exposure to pathogens (biotic stress) (Doke, 1983; Doke and Ohashi, 1988; Montalbini, 1992) then during exposure to abiotic stress (Corpas et al., 1993; Hernández et al., 1993). The mechanisms of production and management of ROS were studied as markers of biotic and abiotic stress, as well through the measurement of enzyme activities.

Tolerance of organisms depends, at least partially, of their ability to cope with ROS. Enzyme activities and proteins such as superoxide dismutases (Cu/Zn SOD, Mn SOD, Fe SOD) (Skórzyńska-Polit et al., 2004; Eyidogan and Öz, 2007; Mobin and Khan, 2007; Wang et al., 2008), catalases (CAT) (Balestrasse et al., 2001; Hsu and Kao, 2004; Cho and Seo, 2005; Mobin and Khan, 2007), ascorbate peroxidases (APX) (Aravind and Prasad, 2003; Mobin and Khan, 2007; Khan et al., 2007; Singh et al., 2008), glutathione reductases (GR) (Skórzyńska-Polit et al., 2004, Khan et al., 2007; Mobin and Khan, 2007; Singh et al., 2008), glutathione transferases (GST) (Dixit et al., 2001; Iannelli et al., 2002; Moons, 2003), glutathione peroxidases (GPX) (Li et al., 2000; Dixit et al., 2001; Leisinger et al., 2001; Avsian-Kretchmer et al., 2004) and metallothioneins (MT) (Anderson et al., 1999; Chiang et al., 2006; Goupil et al., 2009) influence the tolerance of organisms exposed to abiotic stress.

In ecotoxicology, activities of defense enzymes have been widely used as "biomarkers" to assess effects of stress induced by xenobiotics. Indeed, many studies have been conducted in both aquatic and soil ecotoxicology. For example, activity measures of PRX in *Lemna minor* exposed to lead and cadmium (Mohan and Hosetti, 1997), CAT in *L. minor* exposed to folpet and copper (Teisseire et al., 1998), SOD in *Mytilus galloprovincialis* exposed to copper (Gomes et al., 2012), and GR in *Daphnia schoedleri* exposed to hexavalent chromium (Arzate-Cárdenas and Martínez-Jerónimo, 2012) were performed. In soil organisms, measures of defense enzyme activities have been conducted also in soil organisms such as plant and animal species exposed to a large panel of contaminants.

Antioxidant response of organisms has not only been studied at the biochemical level. In most cases, the first level of organization affected by an environmental change (e.g. pollution) is the molecular level, which may lead to changes in gene expression. For more than a decade, molecular biology has led to a better understanding of changes occurring at transcriptional level and allowed acquisition of a large number of very useful genomic and transcriptomic data. Therefore, it is now possible to analyze in detail expression levels of genes belonging to the same physiological function under stress conditions induced by contaminants (Spurgeon et al., 2004; Brulle et al., 2006; Hockner et al., 2009; Bernard et al., 2010; Nakamori et al., 2010; Moudouma et al. (2012)) and some well characterized gene expression variations can be used as "biomarkers of exposure" (Brulle et al., 2006, 2007,

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2008a,b; Vanhoudt et al., 2011a, 2011b; Puckett et al., 2012). However, many organisms are characterized by a lack of molecular data available in databases such as Genbank™. Access to sequence data is crucial for species that are non-model species but are widely used in specific research fields, such as in ecotoxicology (e.g. *Eisenia fetida*, *Helix aspersa*, *Trifolium repens*).

To date, despite numerous studies, it is still very difficult to assess the role of antioxidant system in key species considered as models in soil ecotoxicology. Some species are more favorable because of their biology and eventually the data already available: the oligochaeta annelid *E. fetida* (manure worm) (Łaszczyca et al., 2004; Brulle et al., 2006), the Brassicaceae *Brassica oleracea* (cabbage) (Posmyk et al., 2009; Kusznierewicz et al., 2012) and the Fabaceae *T. repens* (white clover) (Bidar et al., 2009; Manier et al., 2012).

E. fetida is a soil-dwelling organism belonging to the Lumbricidae family. They are considered as soil ecosystem engineers (Lavelle and Spain, 2001) and are good candidate for biomonitoring (Rombke et al., 2005; Sanchez-Hernández, 2006). Indeed, they play an important role in water, nutrients and carbon cycles in terrestrial ecosystems and increase soil fertility (Singh and Pillai, 1973; Edwards and Lofty, 1977; Edwards, 1988). They also provide information on soil structure, microclimatic conditions, nutritional status and presence of toxic elements in soils (Christensen, 1988; Edwards and Bohlen, 1996; Edwards, 1998; Kautenburger, 2006). Annelids live in close contact with the soil through cuticle and gut (Spurgeon et al., 2004; Brulle et al., 2006, Bernard et al., 2010). Therefore, they have been adopted by the international community as sentinel species for the study of the potential environmental impact of anthropogenic contaminants such as pesticides, hydrocarbons and metallic trace elements (MTEs) (Edwards and Bohlen, 1996; Edwards, 1998; Spurgeon et al., 2003; Seeber et al., 2005; Kautenburger, 2006). *E. fetida* is commonly used in soil ecotoxicology and is recommended by OECD for soil contaminants testing since 1984 (OECD, 1984a, and b). Recently, most of the genes encoding oxidative stress protein have been cloned and sequenced (Brulle et al., 2006, 2007, 2008a).

B. oleracea is commonly used in biomonitoring for the accumulation of atmospheric polycyclic aromatic hydrocarbons (PAH) (German standard VDI, 3957, 2000). Mechanisms of accumulation of light organic compounds and their mechanisms of action at the cellular and tissue levels (generation of oxidative stress [MDA contents, APX and GPOX activities], genotoxicity [comet assay]) have been studied (Rzepka, 2007). This species is also used in phytoremediation of metal-contaminated soils (Shahandeh and Hossner, 2002; Kusznierewicz et al., 2012). This plant, which is in close contact with the soil by its roots, is also able to accumulate Zn and Cd in the shoots which have a large leaf area (Kusznierewicz et al., 2012). Measurements of enzyme activities were also conducted in individuals exposed to soil contaminated by MTEs (Hajiboland and Amjad, 2007; Posmyk et al., 2009; Zaimoglu et al., 2011). A partial sequencing of the genome has been performed by Ayele et al. (2005). Thus, a large number of molecular data is now available for this species in different databases. For example, more than 180,000 expressed sequence tags (ESTs) can be found in Genbank™. Nevertheless, great care must be taken with this important amount of data since it has been obtained from different *B. oleracea* varieties. Furthermore, after screening of the entire dataset, few sequences were identified as oxidative stress enzymes and some other were still missing (http://www.ocri-genomics.org/bolbase/; http://brassica.jcvi.org/cgi-bin/brassica/ browse.cgi).

T. repens is a wild, common and ubiquitous species of agronomic interest. Indeed, the white clover is spread over a wide geographical area (sub-arctic zone to sub-tropical regions) and is able to establish a root symbiosis (nodules) with atmospheric nitrogen-fixing bacteria and thus contribute to soil fertilization with nitrogen. As a consequence, *T. repens* is increasingly used in studies focusing on metal-contaminated soils (Bidar et al., 2007, 2009; Manier et al., 2009, 2012). At the beginning of our study (October 2010), there were no sequences encoding oxidative stress proteins in databases for *T. repens* although 40,000 ESTs were produced by Sawbridge et al. (2003) by means of high throughput sequencing.

In this review, we propose a literature survey of the biochemical responses (enzyme activities) and expression variations of genes encoding proteins involved and/or related to antioxidant response in three species that are important in soil ecotoxicology (and in related species). Diverse aspects such as experimental designs, similarities and differences in antioxidant systems between animals and plants and advantages/drawbacks of gene expression and enzyme activity analysis are discussed.

2. Oxidative stress

Organisms exposed to pollution can be more or less damaged depending on matrix, exposure time, type and dose of pollutants in environment. Presence of contaminants may disturb major physiological functions such as survival, nutrition, immunity, growth and reproduction. Disturbance may also be manifested at biochemical level by an imbalance between production and scavenging of ROS generated in presence of pollutants. Oxidative stress, which occurs both in animals and plants, has been extensively studied in numerous species and many contexts (Mascher et al., 2002; Drążkiewicz et al., 2003a,b; Łaszczyca et al., 2004; Lemiere et al., 2005; Brulle et al., 2006; Bidar et al., 2007; Posmyk et al., 2009; Manier et al., 2012). Exposure to xenobiotics can produce many changes both in activity levels and/or gene expression variations of defense enzymes. A literature survey of the biochemical responses (enzyme activities) and expression variations of genes encoding proteins involved and/or related to antioxidant response in Annelids, Brassicaceae and Fabaceae has been done.

2.1. Annelids

Annelids represent a significant part of soil macrofauna biomass (60–80%). As soil engineers, earthworms play a key role in many soil processes (e.g., soil aeration, decomposition of organic matter, soil microbial activity) and are in full contact with the soil (aqueous and solid phase), which makes them relevant indicators of environmental changes (Cortet et al., 1999; Paoletti, 1999; Arnold et al., 2003; Rombke et al., 2005). Contamination may take various routes i.e., ingestion of contaminated soil or food particles, passive adsorption of the metal dissolved in interstitial water through the body wall, ingestion of contaminated soil solution (Arnold et al., 2003). The literature review was carried out on the basis of keywords in Scopus (http://www.scopus.com) and in PubMed (http://www.ncbi.nlm.nih.gov/pubmed) using combinations of the following keywords: '*Eisenia fetida**earthworms*Lumbricidae*enzyme activity* gene expression*xenobiotics*metals*organics*pesticides* aromatic compounds*' in Topics. Publications which appeared relevant for the review were sorted using titles, abstracts and full texts. Concerning gene expression studies, only those using real-time PCR were selected. This procedure allowed us to select a corpus of 51 references (Table 1).

2.1.1. Exposure to MTEs

MTEs are the most studied chemical compounds in terrestrial invertebrates. They are natural components of the Earth's crust which cannot be degraded or destroyed. Some MTEs (e.g. copper (Cu), selenium (Se), zinc (Zn)) are considered as essential elements

Table1
Studies concerning enzyme activities and gene expression measures of candidates related to oxidative stress in Annelida Oligochaeta exposed to metallic and/or organic pollutants.

Medium	Nature of pollutant	Species	Oxidative stress indicator	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
Filter paper	MTEs	*Eisenia fetida*	CAT, GST	Enzyme activity	Cd	48 h	10^{-4}, 0.001, 0.01, 0.1 μg cm^{-2}	↑CAT and GST (10^{-3} μg cm^{-2}).	Zhang et al. (2009b)
Filter paper	MTEs	*Eisenia fetida*	MT, SOD	Gene expression	TiO_2 NPs	24 h	0.1, 1, 10 mg L^{-1}	↑*mt* (× 9.5) and *sod* (× 6.6) (10 mg L^{-1}).	Bigorgne et al. (2011)
Filter paper	MTEs	*Eisenia fetida*	CAT, SOD, GPX	Enzyme activity	ZnO NPs	96 h	0, 50, 100, 200, 500, 1000 mg L^{-1}	↓ SOD (> 100 mg L^{-1}).	Li et al. (2011b)
Filter paper	MTEs	*Eisenia fetida*	MT, CAT, GST, SOD	Gene expression	TiO_2 NPs	4, 12, 24 h	0, 1, 5, 10, 25 μg mL^{-1}	Cœlomocytes exposed in vitro.↑*mt* (4 h, 5 μg mL^{-1} (× 2); 12 h, 10, 25 μg μL^{-1} (× 17, × 22 respectively)).	Bigorgne et al. (2012)
Filter paper	Aromatic compounds	*Eisenia fetida*	CAT, SOD, GST	Enzyme activity, Lipid peroxidation	TBBPA	48 h	0, 0.01, 0.05, 0.1, 0.5, 1 mg L^{-1}	↑ SOD (0.01 mg L^{-1}), CAT (0.01, 0.05 mg L^{-1}) and GST (0.05, 0.1, 0.5 mg.L^{-1}).	Xue et al. (2009)
Filter paper	Aromatic compounds	*Eisenia fetida*	CAT, SOD, PRX	Enzyme activity	B(a)P	48 h	0.001, 0.01, 0.1, 1, 10 mg L^{-1}	↓ SOD and↑PRX (10 mg L^{-1}).	Zhang et al. (2009a)
Filter paper	Aromatic compounds	*Eisenia fetida*	CAT, GST	Enzyme activity	Pyrene	48 h	10^{-4}, 0.001, 0.01, 0.1 μg cm^{-2}	No significant variation.	Zhang et al. (2009a,b)
Filter paper	Aromatic compounds	*Eisenia fetida*	CAT, SOD, PRX	Enzyme activity, Lipid peroxidation	HHCB	1, 2, 3 d	0.1, 1, 10, 100, 200 mg.L^{-1}	↑ SOD (1, 2 d, 100, 200 mg.L^{-1} for both time; 3 d, 10, 100 mg.L^{-1}), CAT (1 d, 200 mg.L^{-1}; 2 d, 100, 200 mg L^{-1}; 3 d, 10, 200 mg L^{-1}) and PRX (1, 2 d, 1 mg L^{-1}; 3 d, 1, 10 mg L^{-1}).	Liu et al. (2010)
Filter paper	Aromatic compounds	*Eisenia fetida*	CAT, SOD	Gene expression, Lipid peroxidation	AHTN, HHCB	12, 24, 36, 48 h	AHTN: 37.5, 375 mg L^{-1}, HHCB: 18.75, 187.5 mg L^{-1}	↑ *sod* (all times, all AHTN and HHCB doses, up to × 1.90).↑*cat* expression levels (AHTN: 37.5 mg L^{-1}, all times; 375 mg L^{-1}, 12, 24 h (up to × 2.57); HHCB: 18.75 mg L^{-1}, 24, 36, 48 h; 187.5 mg L^{-1}, 24 h).↓*cat* and *sod* (48 h, AHTN: 375 mg.L^{-1}, HHCB: 187.5 mg L^{-1}, × 0.52, × 1.37 respectively).	Chen et al. (2011a)
Filter paper	Aromatic compounds	*Eisenia fetida*	CAT, SOD	Gene expression, Lipid peroxidation	AHTN, HHCB	48 h	AHTN: 37.5, 375 mg L^{-1}, HHCB: 18.75, 187.5 mg L^{-1}	↑ *cat* and *sod* (AHTN: 37.5 mg L^{-1}, HHCB: 18.75 mg L^{-1}).↓*cat* and *sod* (AHTN: 375 mg L^{-1}, HHCB: 187.5 mg L^{-1}).	Chen and Zhou (2012)
Filter paper	Pesticides	*Eisenia fetida*	CAT, SOD	Enzyme activity, Lipid peroxidation, Comet assay	Triclosan	48 h	0, 0.5, 2.5, 5, 25, 50 mg L^{-1}	↑ CAT (0.5, 2.5, 5 mg L^{-1}) and SOD (2.5, 5 mg L^{-1}).↓SOD (50 mg L^{-1}).	Lin et al. (2012a)
Artificial soil	MTEs	*Eisenia andrei*	CAT, GPX, GST, NAD (P)H reductases	Enzyme activity, Lipid peroxidation	Pb	2, 7, 14, 28 d	0, 30, 60, 120, 250 mg kg^{-1}	↓ NADPH red (2 d, 60, 120, 250 mg.kg^{-1}), NADH red (2 d, 120 mg kg^{-1}), GST (2 d, all doses) and GR (2 d, 30, 250 mg kg^{-1}; 14 d, 120, 250 mg kg^{-1}).↑NADH red (28 d, 60, 120, 250 mg kg^{-1}).	Saint-Denis et al. (2001)
Artificial soil	MTEs	*Eisenia fetida*	MT, CAT, SOD	Gene expression	Cd	2 h, 6 h, 14 h, 1 d, 2 d, 6 d	80, 800 mg kg^{-1}	↑ *Cd-mt* (80 mg kg^{-1}, 1, 2, 6 d (× 3.45– × 33.33); 800 mg kg^{-1}, 14 h, 1, 2, 6 d (× 7.58– × 205.8)) and *cat* (80 mg kg^{-1}, 2 h (× 1.59); 800 mg kg^{-1}, 6 h (× 1.72)).↓*cat* (14 h, 1 d, 80 and 800 mg kg^{-1} (× 1.3– × 2.22)).	Brulle et al. (2006)
Artificial soil	MTEs	*Eisenia fetida*	MT, CAT, SOD	Gene expression	Cd, Cu	6 h, 14 h, 1 d, 2 d, 6 d, 14 d	Cd: 80, 800 mg kg^{-1}; Cu: 40, 120, 400 mg kg^{-1}	↑ *Cd-mt* (Cd80 (× 5.79– × 63.52) and Cu120 (× 2.46– × 6.07 fold) all times except 6 h; Cd800, all times (× 2.97 to × 85.03); Cu400, 1, 2, 6, 14 d (× 5.29– × 12.49)).↓*cat* (Cd800, 14 h (× 19.23); Cu40, 6 d (× 3.57); Cu400, 2, 6, 14 d (× 2.5– × 2.78)).	Brulle et al. (2007)
Artificial soil	MTEs	*Eisenia fetida*	MT, PCS	Gene expression	Cd	2, 14 d	8, 80, 800 mg kg^{-1}	↑ *Cd-mt* (all doses, all times, × 1.45– × 93.56) and *pcs* (8 mg kg^{-1}, 2, 14 d, × 5.78, × 4.94 respectively).	Brulle et al. (2008a)
Artificial soil	MTEs	*Eisenia fetida*	MT	Gene expression	Cd, Pb, Zn	1, 14 d	40, 500, 700 mg kg^{-1} respectively	↑ *Cd-mt* (14 d, × 5.75)	Brulle et al. (2008b)

Table1 (*continued*)

Medium	Nature of pollutant	Species	Oxidative stress indicator	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
Artificial soil	MTEs	*Lumbricus rubellus*	MT, GST	Gene expression	Cd	28 d	0, 13, 43, 148, 500 mg kg^{-1}	↑ *mt2A*, *mt2B*, *mt2C* (all doses).	Owen et al. (2008)
Artificial soil	MTEs	*Eisenia fetida*	CAT, GST	Enzyme activity	Cd	14, 28 d	0, 50, 10, 200, 400, 800 mg kg^{-1}	↑ CAT (14 d, 400 mg kg^{-1}) and GST (14, 28 d, 100, 200, 400 mg kg^{-1}).↓CAT (28 d, 50 mg kg^{-1}).	Zhang et al. (2009a, b)
Artificial soil	MTEs	*Eisenia fetida*	MT, CAT, SOD	Gene expression	Cu	28 d	Cu NPs: 5, 20, 50 mg kg^{-1} Cu; $CuSO_4$: 2, 10, 20 mg kg^{-1} Cu	↑ *mtl* (Cu NPs, 20, 50 mg kg^{-1} × 3.03, × 3.86 respectively; $CuSO_4$, 10, 20 mg kg^{-1} × 3.73, × 4.49 respectively).	Unrine et al. (2010)
Artificial soil	MTEs	*Eisenia fetida*	MT, CAT	Gene expression	Cd	3 d	0, 5, 25 mg kg^{-1}	↑ *Cd-mt* (× 4, hindgut, 5 mg kg^{-1}; × 18 midgut 1, × 38 hindgut, 25 mg kg^{-1}).↓*cat* (× 1.67, midgut 1, 5 mg kg^{-1}; × 1.43, midgut 1 and hindgut, 25 mg kg^{-1}).	Irizar et al. (2014)
Artificial soil	Aromatic compounds	*Eisenia fetida*	CAT, GST, NAD(P)H reductases	Enzyme activity, Lipid peroxidation	B(a)P	1, 2, 7, 14 d	5×10^{-4}, 1, 100, 1000 mg kg^{-1}	↑ CAT (7 d, 5×10^{-4}, 100, 1000 mg kg^{-1}).↓NADPH red (2 d, 100 mg kg^{-1}) and NADH red (2 d, 100, 1000 mg kg^{-1}; 7 d, 1000 mg kg^{-1}).	Saint-Denis et al. (1999)
Artificial soil	Aromatic compounds	*Lumbricus rubellus*	MT, GST	Gene expression	Fluoranthene	28 d	0, 13.8, 46, 158, 533 mg kg^{-1}	↑ *gstp* (158, 533 mg kg^{-1}).↓*gstp* (13.8, 46 mg kg^{-1}).	Owen et al. (2008)
Artificial soil	Aromatic compounds	*Eisenia fetida*	CAT, GST	Enzyme activity	Pyrene	14, 28 d	0, 40, 80, 160, 640, 1280 mg kg^{-1}	↑ CAT (14 d, 160, 320 mg kg^{-1}) and GST (14 d, 320 mg kg^{-1}).	Zhang et al. (2009a, b)
Artificial soil	Aromatic compounds	*Eisenia fetida*	SOD	Enzyme activity, Lipid peroxidation	Biochar	1, 2, 7, 14 d	200 g kg^{-1}	No significant variation.	Li et al. (2011a,b)
Artificial soil	Aromatic compounds	*Eisenia fetida*	CAT, SOD, GST	Enzyme activity, Lipid peroxidation, Total glutathione	BDE-209	7 d	0, 0.01, 0.1, 1, 5, 10 mg kg^{-1}	↑ SOD (0.01, 0.1 mg kg^{-1}), CAT (1, 5, 10 mg kg^{-1}) and GST (0.1, 0.5 mg kg^{-1}).↓SOD and GST (10 mg kg^{-1}).	Xie et al. (2011)
Artificial soil	Pesticides	*Aporrectodea caliginosa*	GST	Enzyme activity	Chlorpyrifos, Dianizon	1, 2, 4 w	4, 12 mg kg^{-1} respectively	No significant variation.	Booth, O'Halloran (2001)
Artificial soil	Pesticides	*Eisenia andrei*	CAT, GST, GR, NAD(P)H reductases	Enzyme activity, Lipid peroxidation	Carbaryl	2, 7, 14 d	0, 12, 25, 50 mg kg^{-1}	↑ NADH red (14 d, 50 mg kg^{-1}).↓NADPH red (2 d, 25, 50 mg kg^{-1}; 14 d, all doses) and NADH red (2 d, 12 mg kg^{-1}).	Ribera et al. (2001)
Artificial soil	Pesticides	*Lumbricus rubellus*	MT, GST	Gene expression	Atrazine	28 d	0, 9.4, 20.7, 35, 59 mg kg^{-1}	↓ *gst3* (9.4, 20.7 mg kg^{-1}).	Owen et al. (2008)
Artificial soil	Pesticides	*Eisenia fetida*	CAT, SOD, PRX	Enzyme activity, Comet assay	Atrazine	7, 14, 21, 28 d	0, 2.5, 5, 10 mg.kg^{-1}	↑ PRX (2.5, 5 mg kg^{-1}, all times; 10 mg kg^{-1}, 7, 21 d) and CAT (2.5 mg kg^{-1}, 7, 21, 28 d; 5 mg kg^{-1}, 28 d).↓CAT (5 mg kg^{-1}, 7, 14 d; 10 mg kg^{-1}, 14 d) and SOD (2.5 mg kg^{-1}, all times; 10 mg. kg^{-1}, 14, 21 d).	Song et al. (2009)
Artificial soil	Pesticides	*Eisenia fetida*	CAT, SOD, PRX	Enzyme activity, Lipid peroxidation	Formesafen	3, 7, 14, 21, 28 d	0, 10, 100, 500 μg kg^{-1}	↑ PRX (3, 7 d all doses; 14 d, 100, 500 μg kg^{-1}), CAT (3 d, 500 μg kg^{-1}; 7 d, all doses; 14 d, 100, 500 μg.kg^{-1}) and SOD (3 d, 500 μg kg^{-1}; 7 d all doses).↓SOD (14 d, 100, 500 μg kg^{-1}).	Zhang et al. (2012)
Natural spiked soil	MTEs	*Lumbricus rubellus*	MT, GST	Gene expression	Cu	70 d	0, 10, 40, 160, 480 mg kg^{-1}	↑ genes encoding MTs and GSTs with↑[Cu].	Bundy et al. (2008)
Natural spiked soil	MTEs	*Metaphire posthuma*, *Polypheretima elongata*, *Pontoscolex corethurus*	MT-2	Gene expression, Comet assay	Cd	1, 3, 7, 14 d	20, 40, 80 mg kg^{-1}	↑ *mt-2* in all species (*M. posthuma*: up to × 225, *P. corethrurus*: up to × 240, *P. elongata*: up to × 300).	Liang et al. (2011)
	MTEs	*Eisenia fetida*			Ag	1, 3, 7 d			

Table1 (*continued*)

Medium	Nature of pollutant	Species	Oxidative stress indicator	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
Natural spiked soil			MT, ***CAT***, SOD, GST	Gene expression, **Enzyme activity**			$AgNO_3$: 87.9 mg kg^{-1} Ag; Ag NPs: 61.6, 119, 288 mg kg^{-1} Ag	↑ *mt* (3 d, all doses; all times, 288 mg.kg^{-1}, × 3 to × 8) and *gst* (7 d, 61.6, 87.9 mg kg^{-1}, × 2).↓*cat* (Ag NPs, 3 d; 288 mg kg^{-1}, all times, × 2 to × 4) and *sod* (1 d, 61.6 mg kg^{-1}; 3 d, 288 mg kg^{-1}, × 1.5 to × 1.8).↓CAT (87.9 mg kg^{-1}, 3, 7 d; 119 mg kg^{-1}, 3 d).	Tsyusko et al. (2012)
Natural spiked soil	MTEs	*Eisenia fetida*	MT, CAT, SOD	Gene expression	Zn	5, 10, 15 d	62.5, 125, 250, 500 mg kg^{-1}	↑ *CuZnsod* (5 d, 500 mg kg^{-1}; 10 d, 250, 500 mg kg^{-1}; 15 d, 125 mg kg^{-1}; × 2.23 to × 6.22), *cat* (10 d, 250, 500 mg.kg^{-1}; 15 d, 250 mg.kg^{-1}; × 2.51 to × 3.03) and *mt* (10 d, 62.5, 125, 250 mg kg^{-1}; 15 d, 62.5, 250 mg.kg^{-1}; up to × 7.68).↓*cat* (5 d, 250, 500 mg kg^{-1}; × 1.82– × 2.94).	Xiong et al. (2012)
Natural spiked soil	MTEs	*Eisenia fetida*	MT, SOD, ***CAT***, ***PRX***	Gene expression, **Enzyme activity**	Ag	1, 2, 7, 14 d	500 mg kg^{-1}	↑ *Cd-mt* (1 d, Ag NPs, × 1.96).↑CAT(1 d, $AgNO_3$; 7 d, Ag NPs).↓PRX and CAT (2 d, $AgNO_3$).	Hayashi et al. (2013)
Natural spiked soil	MTEs	*Eisenia fetida*	CAT, SOD, GPX	Enzyme activity, Lipid peroxidation	Zn	5, 10, 15 d	0, 125, 250, 500 mg kg^{-1}	↑ CAT and SOD (15 d, 250, 500 mg kg^{-1}) and GPX (5 d, all doses; 15 d, 250, 500 mg kg^{-1}).↓CAT (5 d, 250; 10 d, 500 mg kg^{-1}) and GPX (10 d, 250, 500 mg kg^{-1}).	Xiong et al. (2013)
Natural spiked soil	Aromatic compounds	*Eisenia fetida*	MT, Trx, GST, CAT	Gene expression	TNT	28 d	0, 2, 10.6, 38.7 mg kg^{-1}	↑ *Cd-mt* (× 1.30, 10.6 mg kg^{-1}; × 1.34, 38.7 mg kg^{-1}) and *Trx* (× 1.23, 10.6 mg kg^{-1}).↓*gstp* (× 1.54, 10.6 mg kg^{-1}; × 1.84, 38.7 mg kg^{-1}) and *cat* (× 1.43, 10.6 mg kg^{-1}; × 1.47, 38.7 mg kg^{-1}).	Gong et al. (2007)
Natural spiked soil	Aromatic compounds	*Eisenia fetida*	MT, SOD, GST, PrxR	Gene expression	TNT, RDX	28 d	50, 30 mg kg^{-1} respectively	TNT:↑*PrxR* (× 4.68).↓*Cd-mt* (× 4.62), *sod* (× 2.36) and *gst11* (× 7.80). RDX: No significant variation. TNT + RDX:↓*sod* (× 1.67).	Gong et al. (2008)
Natural spiked soil	Aromatic compounds	*Eisenia fetida*	CAT, SOD, PRX	Enzyme activity, Comet assay	Toluene, Ethylbenzene, Xylene	48 h	Toluene: 5, 10, 50, 100 mg kg^{-1}; Ethylbenzene: 5, 10, 30, 60 mg kg^{-1}; Xylene: 5, 10, 20, 40 mg kg^{-1}	Toluene:↑SOD and CAT (5 mg kg^{-1}). Ethylbenzene:↑CAT (5, 10 mg kg^{-1}) and PRX (5 mg kg^{-1}).↓CAT (15, 30 mg kg^{-1}). Xylene:↑SOD (10 mg kg^{-1}), CAT (all doses) and PRX (5 mg kg^{-1}).	Liu Y. et al. (2010)
Natural spiked soil	Aromatic compounds	*Eisenia fetida*	CAT, SOD	Gene expression	AHTN, HHCB	28 d	AHTN/HHCB: 0, 3, 10, 30, 50, 100 μg g^{-1}	AHTN:↑*cat* and *sod* (30, 50 μg g^{-1}; × 2.7, × 4 and × 1.6, × 2.4 respectively) then↓for both genes (100 μg g^{-1}) (× 2.3, × 1.5 respectively). HHCB:↑*sod* (10, 30, 50, 100 μg g^{-1}; × 1.9, × 3.2, × 3, × 1.5 respectively) and *cat* (30, 50 μg g^{-1}; × 1.8, × 1.9 respectively).	Chen et al. (2011b)
Natural spiked soil	Aromatic compounds	*Eisenia fetida*	CAT, SOD, PRX	Enzyme activity, Lipid peroxidation	HHCB	3, 7, 14, 28 d	0, 10, 50, 100 mg kg^{-1}	↑ SOD (14, 28 d, all doses) and CAT (14 d, 50 mg kg^{-1}; 28 d, 10 mg kg^{-1}).↓SOD (7 d, all doses), CAT (7 d, 50 mg kg^{-1}) and PRX (7, 14 d, 50 mg kg^{-1}; 28 d, 100 mg kg^{-1}).	Liu et al. (2011)
Natural spiked soil	Aromatic compounds	*Lumbricus rubellus*	SOD, ***CAT***, ***GST***	Gene expression, ***Enzyme activity***	Fullerene (C_{60})	4 w	0, 15, 154 mg kg^{-1}	No significant variation in gene expressions and in enzyme activities.	Van Der Ploeg et al. (2013)
Natural spiked soil	Pesticides	*Aporrectodea caliginosa*	CAT, GST	Enzyme activity	Pesticides	3, 7, 14 d	Insecticides (I): 0.011–0.168 mg kg^{-1}; Fungicides (F): 0.025–5.292 mg kg^{-1}	↑ GST (Imax, IFmax) and CAT (Ft, IFmax), 3 d.↓GST (It, Imax, IFmax) and CAT (Imax, Fmax, IFmax), 14 d.	Schreck et al. (2008)
Natural spiked soil	Pesticides	*Eisenia fetida*	CAT, SOD	Enzyme activity, Lipid peroxidation, Comet assay	Triclosan	28 d	0, 0.1, 1, 10, 50, 100 mg kg^{-1}	↑ CAT (10, 50 mg kg^{-1}) and SOD (50, 100 mg kg^{-1}).	Lin et al. (2012a)
Natural spiked soil	Pesticides	*Eisenia fetida*	CAT, SOD, GST	Enzyme activity, Lipid peroxidation, Comet assay	Triclosan	2, 7, 14 d	0, 1, 10, 50, 100, and 300 mg kg^{-1}	↑ SOD (50 mg kg^{-1}), GST (300 mg kg^{-1}) and CAT (100, 300 mg kg^{-1}), 2 d.↓CAT (50, 100, 300 mg kg^{-1}) and GST (100, 300 mg kg^{-1}), 14 d.	Lin et al. (2010)
Natural spiked soil	Pesticides	*Eisenia fetida*	CAT, SOD	Enzyme activity, Comet assay	Chlortetracycline, Tetracycline	7, 14, 28 d	0, 0.3, 3, 30, 100, 300 mg kg^{-1} for both pollutants	Chlortetracycline:↑CAT (3, 100, 300 mg kg^{-1}, 28 d). Tetracycline:↑SOD (3, 30, 300 mg kg^{-1}, 7 d) and CAT (3 mg kg^{-1}, 7 d).↓SOD (30 mg kg^{-1}, 14 d) and CAT (30 mg.kg^{-1}, 28 d).	Dong et al. (2012)

Table1 (*continued*)

Medium	Nature of pollutant	Species	Oxidative stress indicator	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
Natural spiked soil	Pesticides	*Eisenia fetida*	CAT, SOD, GST	Enzyme activity, Lipid peroxidation, Comet assay	Chlortetracycline	28 d	0, 0.3, 3, 30, 100, 300 mg kg^{-1}	↑ SOD (> 3 mg kg^{-1}), CAT (> 3 mg kg^{-1}) and GST (3 mg kg^{-1}).↓GST (> 30 mg kg^{-1}).	Lin et al. (2012b)
Natural spiked soil	MTEs+Organic compounds	*Eisenia fetida*	MT, PCS	Gene expression	Landfill leachates	10 w	400 mL kg^{-1}	↑ *Cd-mt* (100% leachate, × 2.06) and *pcs* (50, 100% leachates, × 3.88, × 2 respectively).	Manier et al. (2012)
Natural polluted soil	MTEs	*Lumbricus terrestris, Aporrectodea caliginosa, Eisenia fetida*	SOD, CAT, GR, GPX, GST	Enzyme activity	MTEs	*in situ* sampling	**Cd**: 0.84–51 mg kg^{-1}, **Pb**: 136–1832 mg kg^{-1}, **Zn**: 151 to 5104 mg kg^{-1}, **Cu**: 10.7–38 mg kg^{-1}.	*A. caliginosa*:↑GPX and GST and↓GR (most polluted site). *E. fetida* / *L. terrestris*:↑GPX (most polluted site).	Łaszczyca et al. (2004)
Natural polluted soil	MTEs	*Eisenia andrei*	CAT, GPX	Enzyme activity, Lipid peroxidation	MTEs	48 h	0.001–12 μg g^{-1}	No significant variation.	Antunes et al. (2008)
Natural pollluted soil	MTEs	*Eisenia fetida*	MT	Gene expression	Cd, Pb, Zn	1, 14 d	15.1, 946, 1191 mg kg^{-1} respectively	↑ *Cd-mt* (14 d, × 10.4)	Brulle et al. (2008b)
Natural polluted soil	MTEs	*Eisenia fetida, Lumbricus rubellus*	MT, PCS	Gene expression	Cd, Pb, Zn	*E. fetida*: into × (2 m), deto × (6 m), reintox (1 m); *L. rubellus*: *in situ* sampling	Polluted soil: 15.1, 946, 1191 mg.kg^{-1} respectively; Reference soil: 0.7, 101, 259 mg kg^{-1} respectively.	*E. fetida:* Intox:↑*Cd-mt* (× 4.71 (6 d) to × 11.18 fold (2 m)). Detox:↓*Cd-mt* (× 10.06 (14 d); × 5.85 (1 m)) then↑*Cd-mt* (2–6 m, × 6.13– × 13.5). Reintox:↑*Cd-mt* (14 d, × 17.59; 1 m, × 19.23). *L.* rubellus:↑*mt* (polluted soil, × 34.39).	Bernard et al. (2010)
Natural polluted soil	MTEs	*Eisenia fetida*	CAT, SOD	Enzyme activity, Lipid peroxidation	Cu	15, 45 d	94–959 mg kg^{-1}	↑ CAT (15, 45 d, 426, 598, 355 mg kg^{-1}) and SOD (45 d, 355–959 mg kg^{-1}).↓CAT (45 d, 959 mg kg^{-1}).	Gaete et al. (2010)
Natural polluted soil	MTEs	*Eisenia fetida*	MT, PCS	Gene expression	MTEs	56 h, 14 d, 56 d	**Cd**: 0.5–21.57 mg kg^{-1}, **Pb**: 32.4–975.56 mg kg^{-1}, **Zn**: 75.8–1242.55 mg kg^{-1}.	Agricultural soils:↑*Cd-mt* (56 d, × 2.18– × 3.83) and *pcs* (56 h, × 1.55– × 2.05).↓*pcs* (14, 56 d) except for 07-2132 and 07-2128 soils. Forest soils:↑*Cd-mt* (07-2134, as soon as 56 h, × 3.09; 07-2135, 14 d, × 2.85; 07-2136, 56 d, × 3.28) and *pcs* (56 h, × 2.72 to × 2.99).↓*pcs* (07-2134, 14 d, × 1.52; 07-2135, 14 d, × 1.24).	Brulle et al. (2011)
Natural polluted soil	MTEs	*Lumbricus terrestris*	GST, GR, GPX	Enzyme activity, Lipid peroxidation, Total glutathione	Hg	2, 44 d	73 to 1287 μg g^{-1}	↑ GST and GPX (44 d, 124 μg g^{-1}).↓GR (2 d, 116 μg g^{-1}) and GPX (44 d, 1287 μg g^{-1}).	Colacevich et al. (2011)
Natural polluted soil	MTEs	*Eisenia fetida*	SOD	Enzyme activity, Comet assay	MTEs	2, 7, 14, 28 d	**Cr:** 65–289 mg kg^{-1}; **Cu:** 58–466 mg kg^{-1}; **Ni:** 34–69 mg kg^{-1}; **Pb:** 57–394 mg kg^{-1}, **Zn:** 238–1729 mg kg^{-1}	↓ SOD (14, 28 d).	Zheng et al. (2013)
Natural polluted soil	MTEs+organic compounds	*Eisenia andrei*	CAT, SOD, GST	Enzyme activity	HMX, MTEs	2, 7, 28 d	**HMX**: 7.3–472.9 mg kg^{-1}, **Bi**: 28.8–184.8 mg kg^{-1}, **Cd**: 8.7–50.7 mg kg^{-1}, **Cr**: 34–317 mg kg^{-1}, **Cu**: 1477.6–13,728.3 mg kg^{-1}, **Ni**: 28.3–397.6 mg kg^{-1}, **Pb**: 245–1963.7 mg kg^{-1}, **Zn**: 245–1334 mg kg^{-1}	↑ CAT (28 d, T2-15, T2-48, T3-19, T3-37 soils) and SOD (28 d, all soils).	Berthelot et al. (2008)

Abbreviations: CAT: catalase, GPX: glutathione peroxidase, SOD: superoxide dismutase, GST: glutathione transferase, MT: metallothionein, NAD(P)H: nicotinamide adenine dinucleotide (phosphate), PRX: peroxidase, PCS: phytochelatin synthase, GR: glutathione reductase, TBBPA: tetrabromobisphenol A, B(a)P: benzo(a)pyrene, HHCB: galaxolide, AHTN: tonalide, BDE-209: decabromodiphenyl ether, MTEs: metal trace elements, HMX: 1,3,5,7-tetranitro-1,3,5,7-tetrazocine, Trx: thioredoxin peroxidase, TNT: trinitrotoluene, RDX: cyclotrimethylenetrinitramine, PrxR: peroxiredoxin.

at low concentrations but can lead to poisoning at higher doses whereas more (e.g. cadmium (Cd), lead (Pb), mercury (Hg)) have no known biological function in most of living organisms and are toxic or poisonous at low concentrations. Human activities, such as smelting ones, lead to accumulation of MTEs in the surrounding soils. MTE poisoning could result, for instance, from drinking-water contamination (e.g. lead pipes), high ambient air concentrations near emission sources or intake via the food chain. A total of 23 relevant references were selected for this topic (Table 1).

2.1.1.1. Enzyme activity. In 2004, Łaszczyca et al. worked on superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione transferase (GST) enzyme activities in whole body of three earthworm species (*Lumbricus terrestris*, *Aporrectodea caliginosa* and *E. fetida*) collected from natural soils contaminated by cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn). The aim of the study was to clarify whether non-specific enzymatic indices were of any diagnostic value in order to determine signs of acclimation of soil fauna to a gradient of metal trace element (MTE) contamination. They observed that GPX activities increased in all species exposed to the most polluted soil (Cd: 51 mg kg^{-1}, Cu: 38 mg kg^{-1}, Pb: 1832 mg kg^{-1}, Zn: 5104 mg kg^{-1}). In *A. caliginosa*, GST activities increased in earthworms exposed to the most polluted soil whereas GR activities decreased. In *E. fetida* and *L. terrestris*, GST and GR activities showed no significant variation. Finally, CAT and SOD activities were not disturbed in all species. In a context of pollution monitoring using these parameters as biomarkers, they postulated that these enzymatic activities variations might be of diagnostic value.

On the contrary, Antunes et al. (2008) did not observe significant variations in GPX and CAT activities in *Eisenia andrei* placed during 48 h in microcosms containing a natural contaminated soil from an abandoned uranium mine. From this experiment, authors concluded that oxidative stress biomarkers did not fully work as sensitive parameters to environmental contamination.

On the other hand, results concerning GR activities in *A. caliginosa* are in accordance with those obtained by Saint-Denis et al. (2001) in *E. andrei* and Colacevich et al. (2011) in *L. terrestris*. The first experiment exposed earthworms in an artificial Pb-spiked soil during 28 days and CAT, GR, GST and NAD(P)H reductases activities were measured. The effect of the different doses of Pb was most apparent when all the responses were analyzed together. According to Saint-Denis et al. this suggests that the suite of biochemical responses of *E. andrei* could be used as sublethal assay for chemical testing in the laboratory or for soil contamination surveys. The second experiment carried out by Colacevich et al. (2011) exposed earthworms in a natural Hg-contaminated soil during 44 days. GST, GR and GPX activities were measured in whole body. Colacevich et al. concluded that GR activities were correlated with reduced glutathione/oxidized glutathione ratio after a 44-day exposure suggesting that GR activities play a key role in cell homeostasis of glutathione.

In 2011, Li et al. studied the impact of ZnO nanoparticles (NPs) on the earthworm *E. fetida*. Organisms were exposed during 96 h either in agar or on filter paper and CAT, SOD and GPX activities were measured in whole body. A slight increase of SOD activities at the lowest exposure dose of ZnO (50 mg kg^{-1}), followed by a decrease at 100 mg kg^{-1} in the agar cubes was observed. This activity is similar to hormesis. SOD activities in earthworms exposed on filter paper was similar. CAT and GPX activities were not significantly influenced in individuals exposed to ZnO NPs whatever NPs concentration and exposure medium.

However, recently, Xiong et al. (2013) observed significant variations in CAT and GPX activities in *E. fetida* exposed to Zn. Indeed, in earthworms exposed to a natural Zn-spiked soil during 15 days, both activities decreased after a 10-d exposure to the highest dose whereas they increased after 5 d of exposure for GPX and 15 d for CAT, respectively. SOD activities were also measured and showed, like CAT, an increase after a 15-d exposure. GPX is the main enzyme to impair the oxidative status during first days then CAT and SOD were the main indicators of oxidative stress for longer times (15 d). According to the authors, the antioxidant enzymatic variations may be an acclimation response of earthworms to survive in contaminated soils.

Another recent study performed by Hayashi et al. (2013) exposed *E. fetida* to a natural soil spiked with $AgNO_3$ or Ag NPs during 2 and 14 days respectively and measured CAT activities. In earthworms exposed to $AgNO_3$-spiked soil, CAT activities increased after 1 d of exposure then decreased after the second day. On the other hand, CAT activities in earthworms exposed to Ag NP-spiked soil showed no significant variation during days 1 and 2 but increased after day 7. *cat* expression levels were also measured in all experimental conditions and no significant variation in gene expression was observed. Similar results concerning CAT activities in earthworms exposed to $AgNO_3$-spiked soil were observed by Tsyusko et al. (2012). They measured CAT activities in whole body of *E. fetida* exposed up to 7 days to a natural soil spiked with Ag NPs or $AgNO_3$. CAT activities decreased after 3 and 7 d of exposure. *cat* expression levels were also measured and showed down-regulations whatever Ag chemical form. According to Tsyusko et al. CAT may not be efficient in degradation of H_2O_2 leading to accumulation of H_2O_2 because of its activity decrease and *cat* down-regulation and an increase in protein carbonyl levels could be noted.

2.1.1.2. Gene expression. In 2006, Brulle et al. cloned and tested expression of 14 potential biomarkers in *E. fetida* following cadmium exposure. Real-Time PCR testing of each candidate was done following exposures to Cd spiked soils. Among these candidates, a cysteine-rich protein involved in metal detoxification, cadmium-metallothionein (*Cd-mt*), and two oxidative stress enzymes (CAT (*cat*) and Cu/Zn SOD (*Cu/Zn sod*)) were studied in immunocytes. *Cd-mt* was significantly induced in *E. fetida* exposed to Cd. This induction was later confirmed in *E. fetida* exposed to field-collected polluted soils (Bernard et al., 2010; Brulle et al., 2011) and also in close related species, *L. rubellus,* collected from polluted sites (Stürzenbaum et al., 2004; Bernard et al., 2010). Concerning oxidative stress enzyme, only *cat* was significantly but transiently induced after a 2-h exposure to soil spiked with 80 mg kg^{-1} Cd and after a 6-h exposure to soil spiked with 800 mg kg^{-1} Cd. A significant decrease of *cat* expression levels was noted (14 h and 1 d for both doses). This work was one of the first antioxidative enzymes study performed at transcriptomic level in earthworms.

One year later, Brulle et al. (2007) performed a similar experiment by adding Cu-spiked soils in their experimental scheme. Then, earthworms were exposed during 14 days to artificial Cd- or Cu-spiked soils and similar effectors to those studied in cœlomocytes were measured in whole bodies after 6 h, 14 h, 1 d, 2 d, 6 d and 14 d of exposure. *Cd-mt* was significantly up-regulated in earthworms exposed to both metal-spiked soils. *cat* expression levels were in accordance with those observed in 2006 in immune cells during Cd exposure. This gene is also down-regulated in earthworms exposed to Cu-spiked soils.

In 2008, by exploiting the conservation observed between species, the same authors cloned the complete coding sequence and characterized at the transcriptional level, the gene coding PCS from the immune cells of *E. fetida* (Brulle et al., 2008a). Expression analysis of *pcs* and *Cd-mt* in animals exposed to artificial Cd-spiked soils during 14 days showed that *pcs* is characterized by a significant increase of its expression level at the lowest cadmium

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concentration. No variation of expression was observed at higher concentrations (80 and 800 mg kg^{-1}). These results suggest that regulation of the gene coding PCS occurs at the transcriptional level in *E. fetida*. *Cd-mt* was characterized by a low but significant induction in worms exposed to 8 mg kg^{-1} whereas higher doses elicited substantially greater inductions. Altogether, these results suggest that *pcs* is involved in dose-dependent Cd detoxification. Furthermore, timing of *pcs* and *Cd-mt* expression in *E. fetida* during Cd intoxication suggests that PCs and Cd-MT may act together in detoxification process, with PCs having an immediate and essential role at low concentration, and Cd-MT participating centrally later and at higher concentrations.

Similar results concerning MT encoding gene expression levels were observed in other earthworm species. For example, *mt2A*, *mt2B* and *mt2C* were up-regulated in *Lumbricus rubellus* exposed to an artificial Cd-spiked soil (Owen et al., 2008) or a natural Cu-spiked soil (Bundy et al., 2008). *mt2* was also up-regulated in *Metaphire posthuma*, *Polypheretima elongata* and *Pontoscolex corethurus* exposed to a natural Cd-spiked soil (Liang et al., 2011).

2.1.2. Exposure to aromatic compounds

Aromatic compounds (a. k. a. arenes) are part of a large class of organic compounds which molecular structure includes one or more planar rings of atoms, usually carbon atoms. Among these compounds, polycyclic aromatic hydrocarbons (PAHs) are a group of chemicals derived from the incomplete combustion of coal, oil, diesel, wood, and other organic materials. There are more than 100 different PAHs (e.g. anthracene, phenanthrene, pyrene, fluoranthene), and they are commonly present in terrestrial and aquatic habitats as complex and diverse mixtures (Alloway and Ayres, 1997). A total of sixteen relevant references were selected for this topic (Table 1).

2.1.2.1. Enzyme activity. In 1999, a study was carried out to elucidate in worms the mechanisms of toxicity of polycyclic aromatic hydrocarbons (PAHs) and to determine whether worm enzymatic responses could be used for monitoring PAH-contaminated soils in laboratory sublethal assays. To do so, Saint-Denis et al. measured CAT and GST activities in whole body of *E. andrei* exposed during 14 days to an artificial soil (OECD, 1984a) contaminated by benzo(a)pyrene (B(a)P). No variation in enzyme activities was observed. These results are in agreement with those obtained by Zhang et al. (2009a and b) who measured CAT, SOD and peroxidases (PRX) activities in whole body of *E. fetida* exposed for 48 h to filter paper contaminated by B(a)P. Increase of SOD and PRX activities was only observed for animals exposed to high doses of B(a)P (10^{-2} mg mL^{-1}).

In 2009, Zhang et al. exposed *E. fetida* to filter paper and artificial soil, both spiked with pyrene, during 48 h and 28 days respectively and CAT and GST activities were measured. No significant variations were observed for both enzyme activities in earthworms exposed during 48 h to filter paper. However, CAT and GST activities increased after a 14-d exposure to pyrene-spiked soil. Earthworms exposed during 28 days to pyrene-spiked soil failed to handle with the high toxicity and were physiologically damaged. Indeed, no survivors were found at 160 and 320 mg kg^{-1} pyrene, indicating the earthworms failed to cope with the toxic stress from exposure to pyrene through their detoxifying and antioxidant mechanisms during a relatively long exposure period.

In 2010, Liu et al. investigated the responses of antioxidant systems after exposure of *E. fetida* to tonalide (HHCB) through contaminated filter paper during 3 days. For SOD and CAT, the activities increased with increasing dose of HHCB, which indicates a dose-dependent pattern. PRX exhibited its peak activity in 1 mg L^{-1} HHCB treatment and decreased at higher concentrations. These two changing patterns were complementary, which reveals the cooperation of enzymes in response to oxidative stress. According to the authors, these results may support the theoretical hypothesis that oxidative stress is an important component for the response of earthworms to the toxicity of HHCB in environment.

One year later, the same team performed a similar experiment by exposing *E. fetida* to a natural HHCB-spiked soil during 28 days (Liu et al., 2011). Among antioxidant enzymes, the primary response to chronic HHCB exposure can be attributed to SOD and CAT. Both activities decreased slightly for short-term exposures (3 and 7 d) then increased for long-term exposures (14 and 28 d), SOD exhibited more sensitive response to HHCB stress. On the other hand, dose-dependent inhibition of PRX activities has been observed whatever exposure duration. According to the authors, SOD and CAT appeared to be the most responsive biomarkers of oxidative stress caused by HHCB in a soil environment.

2.1.2.2. Gene expression. In 2011, Chen et al. exposed *E. fetida* to galaxolide (AHTN) and HHCB in two separated experiments. Earthworms were exposed to contaminated filter paper (Chen et al., 2011a) or to contaminated soil (Chen et al., 2011b). *cat* and *sod* gene expressions were used as biomarkers in whole body of earthworms, to assess AHTN and HHCB adverse effects. Both studies showed a significant increase of *cat* and *sod* expressions during low-dose exposures (AHTN: 0.6 μg cm^{-2} (382.17 mg L^{-1}) and 10 μg g^{-1}/HHCB: 0.3 μg cm^{-2} (191.08 mg L^{-1}) and 10 μg g^{-1} for filter paper and soil exposure, respectively) and a significant decrease of gene expressions during high-dose exposures (AHTN: 6 μg cm^{-2} (3821.66 mg L^{-1}) and 100 μg g^{-1}/HHCB: 3 μg cm^{-2} (1910.83 mg L^{-1}) and 100 μg g^{-1} for filter paper and soil exposure respectively). According to the authors, transcriptional responses of these genes may provide early warning molecular biomarkers for identifying contaminant exposure.

2.1.3. Exposure to pesticides

A pesticide is a chemical or biological agent (such as a virus, bacterium, antimicrobial, or disinfectant) that deters, incapacitates or kills pests. Pesticides fall into five general categories: (i) insecticides, (ii) larvicides, (iii) fungicides, (iv) herbicides, and (v) fumigants. Pesticides are also divided into numerous classes based on chemical nature, of which the most widely reported are organochlorines (e.g., atrazine, dieldrine, 4,4′-DDT) and organophosphorus compounds (e.g., malathion, dimethoate, sulfotepp). A total of 11 relevant references were selected for this topic (Table 1).

2.1.3.1. Enzyme activity. In 2008, Schreck et al. investigated on a vineyard population of *A. caliginosa nocturna* the effects of a mixture of insecticides (λ-Cyhalothrin and Chlorpyrifos-ethyl) and/or fungicides (Folpet, Metalaxyl, Myclobutanil and Fosetyl-Al) at different environmental concentrations. CAT and GST activities were measured on whole body. The aim was to observe the global effects of pesticide exposure, as in a vineyard, rather than focus on each isolated biomarker variation, or on each compound's impact. In total, thirteen microcosms were used: two for control, four for insecticide exposition, four for fungicide exposition and three for insecticide/fungicide mix exposure. Earthworms were collected after 3, 7, 14 and 34 days of exposure. After a 3-day exposure, CAT activities increased significantly in earthworms exposed to insecticides and to pesticide mix. GST activities increased significantly in individuals exposed to fungicides and to pesticide mix. After a 14-day exposure, CAT activities decreased significantly in earthworms exposed to all contaminated conditions. GST activities decreased significantly in individuals exposed to insecticides and to

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pesticide mix. The significant increase in GST and CAT activities revealed the metabolisation of these compounds resulting in the production of reactive oxygen species. After a long period of exposure or high concentrations, earthworms were physiologically damaged: they could not cope with the high toxicity (cellular dysfunction, protein catabolism, etc.).

In 2009, Song et al. exposed *E. fetida* to an OECD artificial soil spiked with atrazine (ATZ) to determine if induction of oxidative stress and DNA damage occurred in earthworms after ATZ exposure. This is a photosynthesis-inhibiting herbicide belonging to a group of chemically similar compounds used to control certain annual broadleaf weeds and grasses. It would have a soil half-life of ~30 days depending on soil type (Alloway and Ayres, 1997). CAT, SOD and PRX activities were measured on whole body and Comet assay was conducted on cœlomocytes. SOD activities were inhibited whatever exposure condition. PRX activities increased in individuals exposed to contaminated soil during 28 days. CAT activities increased in earthworms for low dose exposures and decreased for high dose exposures. Comet assay results showed DNA damage in a time- and dose-dependent manner. Thus, ATZ induces oxidative stress and DNA damage in earthworms.

In 2012, Lin et al. conducted 48 h filter paper contact and 28-day spiked soil tests to study the toxic effects of triclosan (TCS) on the antioxidative and DNA damage indices of *E. fetida* (Lin et al., 2012a). CAT and SOD activities increased significantly for low dose exposure and decreased significantly for high dose exposure. CAT activities results are in agreement with those obtained by Schreck et al. (2008). Important DNA damages in earthworms exposed to TCS-spiked soil during 28 days were observed. A dose-dependence relationship was observed. Two years earlier, the same authors added analysis of GST activities to those of CAT and SOD (Lin et al., 2010). GST activities showed same variations as CAT and SOD activities, i.e., increased significantly for low dose exposure and decreased significantly for high dose exposure. Similar results were observed by the same team (Lin et al. 2012b) and by Dong et al. (2012) in *E. fetida* exposed to chlortetracycline.

2.1.3.2. Gene expression. In 2008, Owen et al. exposed *L. rubellus* to bark-amended loam soils spiked with ATZ during 28 days. Microarray assays were made. Among these assays, *mt2A*, *mt2B*, *mt2C*, *gstα*, *gst3* and *gstp* were studied on whole body. The acute toxicity of ATZ to invertebrates appears to be relatively low, although it is supposed to be carcinogenic and teratogenic in vertebrates; but a striking property is that low concentrations of the herbicide amplifies the toxicities of a number of organophosphate pesticides (Lydy and Linck, 2003), possibly via elevated P450 monooxygenase activity (Anderson and Zhu, 2004). Despite these hazardous properties, only *gst3* was disturbed by ATZ (down-regulation, 9.4 and 20.7 mg kg^{-1} ATZ).

2.1.4. Exposure to mixtures containing MTEs and organic compounds

A total of 2 relevant references were selected for this topic (Table 1).

2.1.4.1. Enzyme activity. In 2008, Berthelot et al. exposed *E. andrei* to soils from military range and training areas, contaminated by HMX (1,3,5,7-tetranitro-1,3,5,7-tetrazocine) and by weapon metals (Zn, Pb, Cd, etc.). The objective was to achieve a bioavailability assessment of soil samples by adopting an integrated approach which combines both chemical and biological tools. Earthworms were exposed to soils during 7 days and CAT, SOD and GST activities were then measured on whole body. GST activities were not affected by metals presence. CAT activities were measurable after 2 days of exposure and inhibited after 7 days. SOD activities increased after 2 days of exposure. According to the authors, elevated superoxide dismutase (SOD) activity suggested that earthworms experienced oxidative stress.

2.1.4.2. Gene expression. In 2012, Manier et al. exposed simultaneously during 10 weeks two test species (*T. repens* and *E. fetida*) to a reference soil contaminated by a landfill leachate (metal and organic compound mixture). *Cd-mt* and *pcs* expression level were measured in cœlomocytes. *Cd-mt* and *pcs* were characterized by an increase in their transcript levels in worms exposed to the reference soil spiked with the leachate, regardless the presence of *T. repens*. These observations support the notion that the timing of PCS and Cd-MT expression in *E. fetida* during Cd intoxication is coordinated.

2.2. Brassicaceae

Brassicaceae are plants belonging to mustards family. *Arabidopsis thaliana*, plant genetic model and *B. oleracea*, our model species, belong to this large family. In this section, a balance of *Arabidopsis* and *Brassica* studies is done. The literature review was carried out on the basis of keywords in Scopus and PubMed using combinations of the following keywords: '*Brassica oleracea**cabbage*Brassicaceae*enzyme activity*gene expression*xenobiotics* metals*organics*pesticides*aromatic compounds*' in Topics. Publications which appeared relevant for the review were sorted using titles, abstracts and full texts. Concerning gene expression studies, only those using real-time PCR were selected. This procedure allowed us to select a corpus of 54 references (Table 2).

2.2.1. Exposure to MTEs

A total of 43 relevant references were selected for this topic (Table 2). Several MTEs were tested in Brassicaceae, Cd and Cu are the most studied. 23 references used *A. thaliana* as test organism whereas twenty preferred *Brassica* species. Only five experimental schemes were conducted on soil matrix.

2.2.1.1. Enzyme activity. Drążkiewicz et al. (2003a) studied APX, GR, dehydroascorbate reductase (DHAR) and monodehydroascorbate reductase (MDHAR) activities in leaves of *A. thaliana* 7-week old plants treated with excess of Cu for 7 days. GR activities were time-dependent whereas those of DHAR were dose-dependent. MDHAR activities increased after a 1-day exposure to 5 μM of Cu then remained important for high Cu concentrations. Finally, APX activities decreased whatever exposure time and copper concentration. One year later, the same authors worked on additional candidates involved in oxidative stress (Drążkiewicz et al., 2004). Briefly, they exposed *A. thaliana* 7-week old plants to Cu solutions during 7 days. SOD, CAT and guaiacol peroxidase (GPOX) activities were measured in leaves. Enzyme activities were disturbed whatever Cu concentrations. SOD and GPOX activities increased whereas CAT activities decreased during Cu exposure.

In 2007, Semane et al. exposed *A. thaliana* 3-week old plants to Cd solutions during one week. APX, GR, CAT, SOD and GPOX activities were measured in leaves. APX and CAT (1 μM), SOD (10 μM) and GR and GPOX (1, 10 μM) activities increased during Cd exposure. Results obtained for APX, GR, SOD and GPOX activities are in accordance with those obtained by Skórzyńska-Polit et al. (2004). Indeed, authors exposed *A. thaliana* 7-week old plants to Cd solutions during 7 days and enzyme activities were measured in leaves. However, a decrease in CAT (all doses) and APX and GPOX (25 μM) activities could be noted.

Two years later, Smeets et al. (2009) proposed a similar experimental scheme with an additional pollutant, Cu. Then, *A. thaliana* 3-week old plants were exposed to Cd and/or Cu

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Table2
Studies concerning enzyme activities and gene expression measures of candidates related to oxidative stress in Brassicaceae and in Fabaceae exposed to metallic and/or organic pollutants.

Family	Medium	Nature of pollutant	Species	Oxidative stress indicator (organ)	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	APX, GR, DHAR, MDHAR (Leaves)	Enzyme activity, Total ascorbate, Total glutathione	Cu	1, 3, 7 d	0, 5, 25, 50, 100 μM	↑ MDHAR (1 d, 5 μM; 3 d, 50, 100 μM; 7 d, 25, 100 μM), DHAR (1 d, all doses; 7 d, 5 μM) and GR (3 d, 25, 50, 100 μM).↓APX (1 d, 5, 25 μM; 3 d, 25 μM; 7 d, 5, 50 μM), DHAR (7 d, 100 μM) and GR (1 d, 25 μM).	Drążkiewicz et al. (2003a)
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	SOD, CAT, GPOX (leaves)	Enzyme activity, ROS content	Cu	7 d	0, 5, 25, 30, 50, 75, 100, 150, 300 μM	↑ SOD and GPOX (all doses).↓CAT (all doses).	Drążkiewicz et al. (2004)
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	CAT, GPOX, SOD, APX, MDHAR, DHAR, GR (Leaves)	Enzyme activity, Total ascorbate, Total glutathione, ROS content	Cd	7 d	0, 5, 25, 50, 100 μM	↑ SOD and GR (all doses), GPOX and APX (5, 50, 100 μM), DHAR (5 μM) and MDHAR (25 μM).↓GPOX and APX (25 μM), CAT (all doses), DHAR (25, 50, 100 μM) and MDHAR (5, 50, 100 μM).	Skórzyńska-Polit et al. (2004)
Brassicaceae	Hoagland solution	MTEs	*Brassica juncea*	SOD, APX, CAT, GR, GST (leaves, roots)	Enzyme activity, Lipid peroxidation	Cr	1, 2, 3, 4, 5, 15 d	0, 0.2, 2, 20 μM	**Leaves:** ↑APX (> 2 d, 0.2, 2 μM; 4, 5 d, 20 μM), CAT (all doses, all times except 0.2 μM, 15 d), GR (> 3 d, 0.2 μM; > 2 d, 2, 20 μM) and GST (4, 5 d, 2 μM).↓APX (15 d, 20 μM). **Roots:** ↑SOD (> 3 d, 0.2 μM; 2, 3, 15 d, 2 μM), APX (2–5 d, 0.2, 2 μM), CAT (> 3 d, 0.2 μM; < 5 d, 2 μM), GR (15 d, 0.2 μM; 2–5 d, 2 μM; > 2 d, 20 μM) and GST (5 d, 0.2 μM; 2–5 d, 2 μM; 3–5 d, 20 μM).↓SOD (4, 5, 15 d, 20 μM) and APX (15 d, 20 μM).	Pandey et al. (2005)
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	SOD (leaves)	Enzyme activity, Total ascorbate, Total glutathione	Cd, Cu	7 d	Cd, Cu: 0, 5, 50 μM	↑ CuZnSOD (Cu, 5, 50 μM), MnSOD and FeSOD (Cd, Cu, 5, 50 μM; FeSOD, 5 μM > 50μM).↓CuZnSOD (Cd, 5, 50 μM)	Drążkiewicz et al. (2007)
Brassicaceae	Hoagland solution	MTEs	*Brassica oleracea*	APX, CAT, PRX, SOD, GR (leaves)	Enzyme activity, Total glutathione	Se	1 w	0, 10, 20 μM	**Cabbage:** ↑GR (10, 20 μM).↓APX and SOD (10, 20 μM). **Kohlrabi:** ↑CAT (20 μM).↓APX and SOD (10, 20 μM).	Hajiboland and Amjad (2007)
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	***APX***, ***GR***, ***CAT***, ***SOD***, ***GPOX***, PCS (leaves)	***Enzyme activity***, Gene expression, Total ascorbate, Total glutathione, PC content	Cd	1 week	0, 1, 10 μM	↑ APX and CAT (1 μM), GR and GST (1, 10 μM) and SOD (10 μM).↑*AtPCS1* (× 2, 10 μM).↓*AtGR1* (× 2, 10 μM).	Semane et al. (2007)
Brassicaceae	Hoagland solution	MTEs	*Brassica napus*	GR (leaves, roots)	Enzyme activity, Total glutathione	Cu	1, 5, 10, 30, 120, 360 min	0, 100 μM	**Leaves:** ↑GR (all times). **Roots:** ↓GR (all times).	Russo et al. (2008)
Brassicaceae	Hoagland solution	MTEs	*Brassica juncea*	GR (whole plant)	Enzyme activity, Total glutathione	Cd	14, 28 d	0, 10, 20, 40, 80, 160 μM	↑ GR (14 d, 40, 80, 160 μM; 28 d, 10, 20 μM). ↓GR (28 d, 160 μM).	Seth et al. (2008)
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	***GPOX***, ***APX***, ***SOD***, ***GR***, ***CAT***, DHAR, GPX (leaves, roots)	***Enzyme activity***, Gene expression	Cd	24 h	0, 5, 10, 20 μM	**Leaves:**↑GPOX (all doses) and APX (5, 10 μM).↓GR (20 μM).↑*AtAPX1* (× 1.4, × 3, × 2) and *AtGR1* (× 2, × 2.2, × 2.25) (all doses), *AtDHAR* and *AtGPX2* (10 μM, × 2.45 and × 2.1) and *AtCAT1* (10 μM, × 3; 20 μM, × 2).↓*AtCSD2* (all doses, × 2.5, × 1.82, × 3.33). **Roots:**↑*AtFSD1* (× 18, × 17.5, × 12.5), *AtCAT1* (× 2, × 2.1, × 2.5) and *AtGR1* (× 4.2, × 3.25, × 3) (all doses), *AtMSD1* (10 μM, × 1.7) and *AtDHAR* (10 μM, × 1.65; 20 μM, × 1.5).↓*AtCSD2* (5 μM, × 1.67; 20 μM, × 1.47).	Smeets et al. (2008)
Brassicaceae	Hoagland solution	MTEs	*Brassica juncea*	PRX (Roots)	Enzyme activity, Lipid peroxidation	Cd	15 d	0, 5, 25, 50, 75, 100, 150, 200 μM	↓ PRX (50, 100 μM).	Verma et al. (2008)
Brassicaceae	Hoagland solution	MTEs	*Brassica juncea*	CAT, SOD, PRX (leaves)	Enzyme activity, lipid peroxidation	Hg	14 d	0, 1, 2, 5, 10, 20 mg.L^{-1}	↑ CAT (2, 10, 20 mg L^{-1}), SOD (10, 20 mg L^{-1}) and PRX (2, 5, 10, 20 mg L^{-1}) activities.	Shiyab et al. (2009)
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	***GPOX***, ***APX***, ***SOD***, ***GR***, ***CAT*** (Leaves, Roots)	***Enzyme activity***, Gene expression, Lipid peroxidation, Total ascorbate, Total glutathione	Cd, Cu, Cd+Cu	24 h	$CdSO_4$, $CuSO_4$: 0, 10 μM	**Leaves:** ↑*AtCSD1* (× 3.36, × 4.42) and *AtMSD1* (× 1.63, × 2.08) (Cu, Cd+Cu), *AtAPX1* (× 2.56, × 2.51, × 2.73) and *AtGR1* (× 2.56, × 1.83, × 4.28) (all conditions), *AtCAT1* (Cd, × 2.29; Cu, × 2.04) and *AtFSD1* (Cd+Cu, × 6.16).↓*AtCSD2* (Cd, × 3.23; Cd+Cu, × 1.27) and *AtFSD1*	Smeets et al. (2009)

Table2 (*continued*)

Family	Medium	Nature of pollutant	Species	Oxidative stress indicator (organ)	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
									(Cu, × 5.26).↑GPOX, CAT and GR (Cd) and APX (Cd, Cd+Cu). **Roots:** ↑*AtFSD1*(× 5.88), *AtCAT1*(× 2.14), *AtAPX1* (× 1.83) and *AtGR1*(× 5.90) (Cd).↓*AtCSD1* (× 7.14, × 12.5), *AtCSD2* (× 20, × 14.29), *AtFSD1* (× 500, × 33.33), *AtMSD1* (× 11.11, × 10), *AtAPX1* (× 20, × 8.33) and *AtGR1* (× 14.29, × 4.55) (Cu, Cd+Cu) and *AtCAT1* (Cu, × 1.09).↑GPOX (Cd, Cd+Cu).↓CAT and APX (Cu) and GR (Cd, Cd+Cu).	
Brassicaceae	Hoagland solution	MTEs	*Brassica juncea*	SOD, CAT, APX, GPOX (leaves, Roots)	Enzyme activity	Cu	3, 7, 14 d	0, 10, 50, 100, 200 μM	**Leaves:** ↑SOD (7 d, 100, 200 μM; 14 d, all doses), CAT (3 d, 100, 200 μM; 7 d, 50, 100, 200 μM; 14 d, all doses), APX (7, 14 d, all doses) and GPOX (7 d, 200 μM; 14 d, all doses).↓SOD (3 d, 10 μM). **Roots:** ↑SOD (3 d, 10, 50, 100 μM; 7 d, 100, 200 μM; 14 d, all doses), CAT (3 d, 50, 100, 200 μM; 7, 14 d, all doses) and APX and GPOX (all times, all doses).↓SOD (3 d, 200 μM).	Singh et al. (2010)
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	***APX***, ***SOD***, ***GR***, ***CAT*** (leaves, Roots)	***Enzyme activity***, Gene expression, Lipid peroxidation, Total ascorbate, Total glutathione	Cd, Cu	24 h	Cd: 0, 5, 10 μM; Cu: 0, 2, 5 μM	**Leaves:** ↑APX (Cd), CAT and GR (Cu5).↑*AtGR1* (× 208, × 215) and *AtCAT1* (× 226, × 320) (Cd), *AtCAT3* (Cd10, × 203; Cu5, × 417) and *AtAPX1* (Cd10, × 266; Cu2, × 146).↓*AtCSD2*(× 7, × 10), *AtCSD3* (× 27, × 41) and *AtCAT2* (× 18, × 27) (Cd), *AtFSD1* (Cd5, × 56; Cu5, × 15), *AtFSD2* (Cd, × 14, × 16; Cu 5, × 50) and *AtFSD3* (Cd, × 14, × 27; Cu, × 51, × 66). **Roots:** ↑CAT and GR (Cd 5).↓CAT (Cu5) and APX (Cu).↑*AtCSD2* (Cu, × 150), *AtFSD1* (Cd, × 15, × 3.33), *AtMSD1* (Cd10, × 165), *AtCAT1* (Cd10, × 534; Cu2, × 640), *AtCAT3* (Cd10, × 261; Cu, × 743, × 335), *AtAPX2* (Cd, × 587, × 6372; Cu, × 261, × 374) and *AtGR1* (Cd, × 422, × 322).↓*AtCSD1* (× 46) and *AtCAT2* (× 56) (Cd5), *AtCSD3*(× 69, × 47) and *AtFSD3* (× 48, × 43) (Cd), *AtCSD2* (Cd5, × 56), *AtFSD2* (Cd, × 58, × 37; Cu, × 61, × 48), *AtMSD1* (Cu5, × 14) and *AtAPX1* (Cu, × 28, × 7).	Cuypers et al. (2011)
Brassicaceae	Hoagland solution	MTEs	*Brassica napus*	APX, (M) DHAR, GR, GST, GPX, CAT (Seedlings)	Enzyme activity, Lipid peroxidation, Total ascorbate, Total glutathione	Cd, Se	24 h (Se) then 48 h (Cd)	Cd: 0, 0.5, 1 mM; Se: 0, 50, 100 μM	↑ APX (Se50, Cd0.5+Se, Cd1+Se50), MDHAR (Cd+Se), DHAR (Cd0.5+Se), GR (Cd0.5, Cd+Se), GPX (Cd0.5, Se, Cd+Se), GST (Cd, Se100) and CAT (Cd0.5+Se, Cd1+Se100).↓MDHAR, DHAR and CAT (Cd), GR and GPX (Cd1).	Hasanuzzaman et al. (2012)
Brassicaceae	Hoagland solution	MTEs	*Brassica juncea*	PRX, CAT (leaves, roots)	Enzyme activity, Lipid peroxidation, Total ascorbate, Total glutathione, PC content	Cd	7 d	0, 50, 200 μM	**Leaves:** ↑PRX (200 μM).↓CAT (50, 200 μM). **Roots:** ↑PRX (200 μM).	Mohamed et al. (2012)
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	CAT, APX, SOD (Leaves, Roots)	Gene expression, Lipid peroxidation	Cd, Cu	2, 4, 6, 24 h	Cd: 0, 5 μM; Cu: 0, 2 μM	**Leaves:** ↑*AtCSD1* (Cu, 6, 24 h, × 1.44, × 4.38), *AtCSD2* (Cu, 24 h, × 1.73) and *AtCAT3* (Cu, Cd, 24 h, × 2.80, × 1.48).↓*AtFSD1* (Cu, 6, 24 h, × 2.38, × 43.48) and *AtCAT2* (Cd, 24 h, × 1.27). **Roots:** ↑*AtCSD1* (Cu, × 1.67, × 2.02, × 2.17, × 2.90), *AtCSD2* (Cu, 24 h, × 2.15), *AtFSD1* (Cd, 24 h, × 4.24), *AtAPX1* (Cu, 4 h, × 1.73) and *AtCAT1* (Cu, 4, 6, 24 h, × 2.57, × 3.74, × 16.64; Cd, 24 h, × 3.43).↓*AtCSD1* (× 2.13) and *AtCSD2* (× 2.33) (Cd, 24 h), *AtFSD1* (Cu, 6, 24 h, × 3.13, × 83.33), *AtCAT2* (Cu 2, 4, 24 h, × 3.03, × 2.86, × 2.94) and *AtCAT3* (Cu, 2, 4 h, × 3.13, × 2.44).	Opdenakker et al. (2012)

Table2 (*continued*)

Family	Medium	Nature of pollutant	Species	Oxidative stress indicator (organ)	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
Brassicaceae	Hoagland solution	MTEs	*Arabidopsis thaliana*	***SOD***, ***CAT***, ***APX***, ***GPOX***, ***GR***, PCS (leaves, Roots)	***Enzyme activity***, Gene expression, Total glutathione, PC content	Zn	24 h	0, 100, 250, 500 μM	**Leaves:**↑GPOX (all doses), CAT (100, 250 μM) and SOD (250 μM).↓GR (250, 500 μM).↑*AtGR1* (all doses, × 2.03, × 1.88, × 1.68) and *AtCSD1* (100, 250 μM, × 2.30, × 1.70).↓*AtGR2*(× 1.82, × 1.72, × 1.49), *AtCAT2*(× 2.63, × 1.67, × 2.63), *AtFSD2* (× 4, × 3.33, × 5), *AtCSD2* (× 2.5, × 3.33, × 10) and *AtFSD3* (× 3.23, × 2.56, × 3.85) (all doses) and *AtFSD1* (100, 250 μM, × 2.78, × 2.63). **Roots:**↑GPOX (100, 250 μM) and CAT (250, 500 μM).↓GR (250, 500 μM) and SOD (250 μM).↑*AtGR1* (250, 500 μM, × 1.51, × 1.50), *AtCAT1* (× 9.89, × 10.57, × 14.22) and *AtAPX2* (× 12.62, × 29.77, × 141.18) (all doses), *AtCAT3* (100 μM, × 2.08), *AtCSD2* (100, 250 μM, × 1.45, × 1.3) and *AtFSD1* (500 μM, × 2.82).	Remans et al. (2012)
Brassicaceae	Hoagland solution	Aromatic compounds	*Brassica campestris*, *Brassica rapa*	CAT, SOD, GPOX, GR, APX (Leaves)	Enzyme activity, Lipid peroxidation	Phenanthrene	2 w	0, 30, 100, 300 μM	***B. campestris:***↑SOD, GPOX, CAT, APX and GR (all doses). ***B. rapa:***↑GPOX, CAT and APX (all doses) and GR (300 μM).↓SOD (all doses) and GR (30 μM).	Ahammed et al. (2012)
Brassicaceae	Hoagland solution	Pesticides	*Brassica juncea*	GST, CAT, APX, GPOX, GR (Leaves, Roots)	Enzyme activity	Acetaminophen	1, 3, 7 d	1 mM	**Leaves:**↑GST and GPOX (all times) and APX (7 d).↓CAT (7 d) and GR (1, 3 d). **Roots:**↓GST and GR (all times) and CAT, APX and GPOX (7 d).	Bartha et al. (2010)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	CAT, SOD, APX, GR, GPOX (Seedlings)	Enzyme activity, Lipid peroxidation	Cd	21 d	0, 300, 500 μM	↑ APX (all doses) and GPOX (300 μM).↓SOD, CAT and GR (all doses) and GPOX (500 μM).	Cho and Seo (2005)
Brassicaceae	Other medium	MTEs	*Arabidopsis halleri*, *Arabidopsis thaliana*	***CAT***, ***APX***, ***PRX***, MT, MDHAR (seedlings)	***Enzyme activity***, Gene expression	Cd	3, 24 h	0, 50, 100 μM	*AhMT2A* > *AtMT2A* (up to × 4.13), *AhMT2B* > *AtMT2B* (up to × 4.33), *AhMT3* > *AtMT3* (up to × 2.40), *AhAPX1* > *AtAPX1* (× 2.80), *AhAPX3* > *AtAPX3* (× 2.10) and *AhMDAR4* > *AtMDAR4* (× 2.10). CAT Ah > At (× 1.09), APX Ah > At (× 6.99) and PRX Ah > At (× 15.97).	Chiang et al. (2006)
Brassicaceae	Other medium	MTEs	*Arabidopsis halleri*, *Arabidopsis thaliana*	***CAT***, ***APX***, ***PRX***, MT, MDHAR (seedlings)	***Enzyme activity***, Gene expression	Cd	3, 24 h	0, 50, 100 μM	*AhMT2A* > *AtMT2A* (up to × 4.13), *AhMT2B* > *AtMT2B* (up to × 4.33), *AhMT3* > *AtMT3* (up to × 2.40), *AhAPX1* *AtAPX1* (× 2.80), *AhAPX3* > *AtAPX3* (× 2.10) and *AhMDAR4* > *AtMDAR4* (× 2.10). CAT Ah > At (× 1.09), APX Ah > At (× 6.99) and PRX Ah > At (× 15.97).	Chiang et al. (2006)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	SOD, CAT, APX (Leaves)	Enzyme activity	Cd, Cu	1, 5, 15 h	100 μM	↑ CAT (Cd, 15 h), APX (Cu, 1, 15 h; Cd, 15 h) and SOD (Cd, 1 h).↓CAT (Cu, 15 h), APX (Cu, 5 h) and SOD (Cu, Cd, 5 h).	Maksymiec and Krupa (2006)
Brassicaceae	Other medium	MTEs	*Brassica chinensis*	SOD, CAT, APX, GPOX (Leaves)	Enzyme activity, Lipid peroxidation	Cd	6 d	0, 5, 25, 50, 100, 200 μM	↑ CAT and APX (all doses) and GPOX (> 50 μM).	Chen et al. (2008)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	APX, GR, SOD, AOX, GLR, PrxR, MDHAR, Trx, PRX, GST (Roots)	Gene expression	Al	6, 48 h	0, 25 μM	↑ *AtMDAR6* (× 2.39), *AtRHS19* (× 3.68), *AtPER64* (× 2.07), *AtPRXR1* (× 1.34), *AtPRX1* (× 7.26), *AtGSTU17* (× 4.76) and *AtGSTU27* (× 2.57) (6 h) and *AtAPX2* (× 15.67), *AtGR1* (× 18.90), *AtFSD1* (× 1.57), *AtMDAR3* (× 2.27), *AtPDI12* (× 1.59), *AtTrx3* (× 1.79), *AtGSTU19* (× 1.87) and *AtGSTU17* (× 42.22) (48 h).↓*AtAOX1A* (× 1.79), *AtPRX34* (× 1.82), *AtGSTU8* (× 7.26), and *AtGSTU22* (× 9.71) (6 h) and *AtAOX2* (× 91.14), *AtGLRS13* (× 1.53), *AtMDAR2* (× 3.36), *AtNTR1* (× 8.46) and *AtGSTU9* (× 2.28) (48 h).	Kumari et al. (2008)
Brassicaceae	Other medium	MTEs	*Brassica oleracea*	SOD, CAT, GPOX, APX, GPX, GR (Seedlings)	Enzyme activity, Lipid peroxidation	Cu	1, 2, 3, 4 d	0.5, 2.5 mM	↑ SOD and APX (2.5 mM, all times), CAT (0.5 mM, all times), GPOX (all doses, all times) and GPX (2.5 mM, 1 d).↓CAT (2.5 mM, all times), GR (all doses, all times) and GPX (all doses, > 2 d).	Posmyk et al. (2009)
Brassicaceae	Other medium	MTEs	*Brassica juncea*	GST (Seeds)	Enzyme activity, Lipid peroxidation, Total glutathione	Cd	12, 24, 48, 96 h	0, 50, 100, 200 mg L^{-1}	↑ GST (200 mg.L^{-1}, 24 h).	Szőllősi et al. (2009)

Table2 (*continued*)

Family	Medium	Nature of pollutant	Species	Oxidative stress indicator (organ)	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	CAT, SOD (Leaves, Roots)	Gene expression	U	3 d	0, 0.1, 1, 10, 100 μM	**Leaves:**↑*AtCAT1* (1, 10, 100 μM, × 2.40, × 3, × 1.50).↓*AtCSD1* (10, 100 μM, × 2, × 3.33). **Roots:**↑*AtMSD1* (× 1.31) and *AtFSD1* (× 3) (100 μM) and *AtCAT1* (10, 100 μM, × 1.67, × 2.11).↓*AtCSD1* (10, 100 μM, × 1.23, × 1.16).	Vanhoudt et al. (2009)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	CAT, SOD (Leaves, Roots)	Gene expression, Lipid peroxidation	U	3 d	10 μM	**Leaves:**↓*AtCAT1* (× 2), *AtCAT3* (× 1.67), *AtCSD1* (× 3.33) and *AtCSD2* (× 4). **Roots:**↓*AtCSD2* (× 2.5), *AtCSD3* (× 2) and *AtFSD2* (× 2).	Vanhoudt et al. (2010a)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	CAT, SOD, APX, GR (Leaves, Roots)	Gene expression, Lipid peroxidation	U, Cd	3 d	U: 10 μM; Cd: 5 μM	**Leaves:**↓*AtCSD3* (Cd, × 3.33), *AtCSD1* (× 2.22, × 3.33, × 10), *AtCSD2* (× 2.86, × 8.33, × 20), *AtFSD2* (× 2.08, × 5.26, × 4) and *AtCAT2* (× 3.33, × 8.33, × 6.67) (U, Cd, U+Cd) and *AtFSD1* (× 14.29, × 8.33), *AtFSD3* (× 4, × 2.5), *AtMSD1* (× 2.86, × 2.17), *AtAPX1* (× 6.67, × 10), *AtCAT1* (× 4.55, × 5), *AtCAT3* (× 5.56, × 3.33) and *AtGR1* (× 2.86, × 3.57) (Cd, U+Cd). **Roots:**↑*AtFSD1* (Cd, × 1.60; U+Cd, × 2.45).↓*AtCAT1* (Cd, × 2.86), *AtCSD1* (× 5) and *AtFSD2* (× 2.86) (U+Cd) and *AtCSD2* (× 2.22, × 4) and *AtCAT2* (× 2.50, × 3.33) (Cd, U+Cd).	Vanhoudt et al. (2010b)
Brassicaceae	Other medium	MTEs	*Brassica juncea*	CAT, PCS (Seedlings)	Gene expression	Cd, Pb	7 d	Cd: 0.15 M; Pb: 1 M	↑ *BjPCS1* (Cd, × 1.67; Pb, × 2) and *BjCAT3* (Cd, × 2.4; Pb, × 1.6).	Bhuiyan et al. (2011)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	PCS (Seedlings)	Gene expression, Total glutathione, PC content	Cd	5, 9 d	30 μM	**5 d:** *AtPCS1* wild-type < *AtPCS1* AtPCSox-4 (× 1.38) and AtPCSox-5 (× 1.31). **9 d:** *AtPCS1* wild-type < *AtPCS1* AtPCSox (× 1.19 to × 1.81).	Brunetti et al. 2011
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	SOD, CAT, GR, GPX, PRX (Seedlings)	Enzyme activity	Pb	7 d	0, 100 μM	↑ SOD, CAT, GR, GPX and PRX.	Phang et al. 2011
Brassicaceae	Other medium	MTEs	*Brassica campestris*	PRX (Roots)	Enzyme activity	Pb	24 h	0, 50, 100, 250, 500 μM	↑ PRX (all doses).	Singh et al. (2011)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	CAT, SOD, APX, GR (Roots)	Gene expression, Total glutathione, Total ascorbate	U	1, 3, 7 d	0, 0.1, 1, 10, 100 μM	↑ *AtFSD1* (100 μM, 1 d, 3 d, × 5, × 4), *AtMSD1* (× 1.29) and *AtAPX1* (× 1.36) (100 μM, 3 d), *AtCAT1* (10, 100 μM, 1 d, x 1.3, x 6.9) and *AtGR1* (100 μM, 3 d, 1.35).↓*AtCSD1* (1 d, 100 μM, × 2; 3 d, 10, 100 μM, × 1.25), *AtCSD2* (1 d, 1, 10, 100 μM, × 1.22, × 1.25, × 3.33; 3 d, 100 μM, × 1.45) and *AtGR1* (1 d, 10 μM, 1.54; 3 d, 1, 10 μM, × 1.56, × 1.82).	Vanhoudt et al. (2011a)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	CAT, SOD, APX, GR (Leaves)	Gene expression, Total glutathione, Total ascorbate	U	1, 3, 7 d	0, 0.1, 1, 10, 100 μM	↑ *AtCAT1* (3d, 1, 10 μM, × 2.58, × 3.33; 7 d, 1 μM, × 3.29).↓*AtAPX1* (3 d, 100 μM, × 1.61), *AtCSD1* (1 d, 100 μM, × 6.67; 3 d, 10, 100 μM, × 2.30, × 3.70), *AtCSD2* (1 d, 100 μM, x 10; 3 d, > 1 μM, × 1.92, × 4.35, × 12.5; 7 d, 100 μM, × 4), *AtCAT2* (3 d, 100 μM, x 12.5; 7 d, 10, 100 μM, × 10, × 20) and *AtGR1* (1 d, 10, 100 μM, × 1.67, × 2.38; 3 d, > 0.1 μM, × 1.54, × 2.86, 3.45, × 5; 7 d, > 1 μM, × 3.7, × 5, × 4.76).	Vanhoudt et al. (2011b)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	***PCS*** (Leaves, Roots)	***Enzyme activity***, Gene expression, PC content, Proline content	Cd	1 week	100 μM	**Leaves:**↑PCS, *AtPCS1* (× 2.43) and *AtPCS2* (× 3.75). **Roots:**↑PCS, *AtPCS1* (× 1.05) and *AtPCS2* (× 3.54).	Moudouma et al. (2012)
Brassicaceae	Other medium	MTEs	*Arabidopsis thaliana*	GST, SOD, PRX (Whole plant)	Gene expression	Ag	24 h	**$AgNO_3$/Ag NPs:** 5 mg L^{-1}	↑ *At2g38390* (x 4.55, $AgNO_3$; x 2.63, Ag NPs), *AtGSTF12* (× 4.17, $AgNO_3$), *AtGSTU22* (× 3.85, $AgNO_3$), *AtCSD2* (× 3.33, $AgNO_3$), *At1g14550* (× 3.23, $AgNO_3$), *AtGSTU25* (× 3.23, $AgNO_3$; × 2.75, Ag NPs), *AtCSD1* (× 3.03, $AgNO_3$; × 3.63, Ag NPs), *At3g21770* (x 2.86, $AgNO_3$), *AtGSTU8* (× 2.56, $AgNO_3$; × 2.78, Ag NPs), *At2g18150* (× 2.50, $AgNO_3$), *At3g01190* (× 2.44, $AgNO_3$), *At5g51890* (× 2.44, $AgNO_3$; × 2.69, Ag NPs), *AtGSTU6* (× 2.38,	Kaveh et al. (2013)

Table2 (*continued*)

Family	Medium	Nature of pollutant	Species	Oxidative stress indicator (organ)	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
									$AgNO_3$; × 2.50, Ag NPs), *AtGSTU20* (× 2.22, $AgNO_3$), *At5g14130* (× 2.08, $AgNO_3$; × 2.73, Ag NPs) and *AtPRX53* (× 3.83, Ag NPs).↓*AtGSTF3* (× 2.08, $AgNO_3$) and *AtFSD1* (× 2.27, $AgNO_3$; × 2.5, Ag NPs).	
Brassicaceae	Other medium	MTEs	*Brassica napus*	***SOD***, ***APX***, ***GPOX***, ***CAT***, MT (leaves)	***Enzyme activity***, Gene expression, Lipid peroxidation	Cr	1, 7 d	50 μM	↑ SOD and GPOX (1, 7 d).↓APX (1, 7 d) and CAT (7 d).↑*BnMP1* (1 d).↓*BnMP1* (7 d)	Yildiz et al. (2013)
Brassicaceae	Other medium	Aromatic compounds	*Sinapsis alba*	GST, GPX, GR (Seeds)	Enzyme activity	PAHs	96 h	0, 0.02, 0.2, 2, 20, 200 μM	↑ GST, GPX and GR.	Paskova et al. (2006)
Brassicaceae	Other medium	Aromatic compounds	*Arabidopsis thaliana*	GST, CAT, APX, SOD (Seedlings)	Gene expression	Phenanthrene	21 d	0, 0.25, 0.5 mM	↑ *AtGSTU24* (× 4.28), *AtGSTZ1* (× 3.03), *AtGSTF3* and *AtGSTF4* (× 3.73), *AtGSTF6* and *AtGSTF7* (× 12.04), *AtCSD1* (× 4.92) and *AtCSD2* (× 3.03).↓*AtAPX4* (× 2.83), *AtFSD1* (× 16), *AtCAT1* (× 1.46), *AtCAT2* (× 1.55), *AtCAT3* (× 1.34) and *AtTAPX* (× 4.44).	Weisman et al. (2010)
Brassicaceae	Other medium	Aromatic compounds	*Arabidopsis thaliana*	***GST***(Whole plant, Roots, Cell cultures)	***Enzyme activity***, Gene expression	CDNB	24 h	100 μM	↑ GST (all samples).↑*AtGSTU19* (× 1.16, Plant; × 5.71, Roots; × 4.63, Cells) and *AtGSTU24* (× 2.76, Plant; × 24.22, Roots; × 34.46, Cells).	Skipsey et al. (2011)
Brassicaceae	Other medium	Pesticides	*Arabidopsis thaliana*	SOD, CAT, GPOX, APX (Whole plant)	Enzyme activity	Colchicine	3 weeks	2 mg L^{-1}	↑ SOD.↓CAT, APX, GPOX.	Drążkiewicz et al. (2003b)
Brassicaceae	Other medium	Pesticides	*Arabidopsis thaliana*	APX (Leaves)	Gene expression	Paraquat	4 h	20 μM	↑ *AtAPX1* (× 9).	Laloi et al. (2007)
Brassicaceae	Other medium	Pesticides	*Arabidopsis thaliana*	APX, GST (Seedlings)	Gene expression	Atrazine	48 h	10 μM	↑ *AtGSTU11* (× 13.27).↓*AtAPX1* (× 3.53) and *AtGSTU20* (× 13.18).	Ramel et al. (2007)
Brassicaceae	Other medium	Pesticides	*Arabidopsis thaliana*	GST (cell cultures)	Gene expression	Fenclorim	60 min	100 μM	↑ *AtGSTU19* (× 4, 40 min; × 9, 60 min), *AtGSTU24* (× 80, 40 min; × 250, 60 min) and *AtGSTF8* (× 3, 40 min; × 5, 60 min).	Skipsey et al. (2011)
Brassicaceae	Other medium	Pesticides	*Arabidopsis thaliana*	APX, CAT, SOD (cell cultures)	Gene expression, total glutathione	Paraquat	1 h	10 μM	↑ *AtCAT1* (up to × 3.38), *AtAPX1* (up to × 3.44), *AtAPX2* (up to × 3.14), *AtAPX3* (up to × 2.64) and *AtCSD1* (up to × 1.94).	Bulgakov et al. (2012)
Brassicaceae	Soil	MTEs	*Brassica juncea*	CAT, SOD, APX, GR (leaves)	Enzyme activity, Lipid peroxidation	Cd	30 d	0, 25, 50, 100 mg kg^{-1}	↑ CAT, SOD and APX (all doses, Varuna, RH-30) and GR (all doses, Varuna; 25, 50 mg kg^{-1}, RH-30).↓GR (100 mg kg^{-1}, RH-30).	Mobin and Khan (2007)
Brassicaceae	Soil	MTEs	*Brassica juncea*	CAT, APX, GPOX, SOD (leaves, roots)	Enzyme activity, Lipid peroxidation	Cd	90 d	0, 5, 15, 35 mg kg^{-1}	**Leaves:**↑CAT and APX (15, 35 mg kg^{-1}) and GPOX (all doses). **Roots:**↑APX and GPOX (35 mg kg^{-1}).	Pinto et al. (2009)
Brassicaceae	Soil	MTEs	*Brassica juncea*	SOD, APX, GPOX, GR (whole plant)	Enzyme activity	Cu, Cr, As(III), As(V)	15, 30 d	Cr, As: 10, 30, 50 μg g^{-1}; Cu: 30, 50 μg g^{-1}	↑ APX (15 d, Cu30, As(V)50; 30 d, As(III)10) and GPX (15d, As10, 30; 30 d, Cr50).↓SOD (15 d, As(III)10, 30, As (V); 30 d, Cu50, As(V)30, 50, Cr30, 50), GR (Cu50, As(V), Cr; 30 d, Cu, As(V)10, 30, Cr) and APX (30 d, As(V)50, Cr).	Sinha et al. (2010)
Brassicaceae	Soil	MTEs	*Brassica oleracea*, *Brassica juncea*	CAT, APX, GR (leaves)	Enzyme activity	Cr	10 weeks	0, 0.05, 0.25, 0.5, 1 mM	↑ APX (*B. oleracea*, 0.05, 0.25 mM; *B. juncea*, 0.05, 0.25, 0.5 mM) and GR (Both species, 0.05, 0.25 mM).↓APX (*B. oleracea*, 1 mM) and CAT (both species, > 0.05 mM).	Zaimoglu et al. (2011)
Brassicaceae	Soil	MTEs	*Brassica juncea*	***SOD***, ***APX***, ***CAT***, ***GR*** (Whole plant)	***Enzyme activity***, Gene expression, Lipid peroxidation	Cd	45 d	20 mM	↑ SOD, APX, CAT and GR.↑*BjSOD* (× 2.5), *BjCAT* (× 2.4), *BjAPX* (× 3) and *BjGR* (× 2.25).	Kumar et al. (2013)
Brassicaceae	Soil	Pesticides	*Brassica campestris*	SOD, PRX, CAT, GR (whole plant)	Enzyme activity	TCA	10 d	0, 0.48, 1.90, 3.90, 7.80, 15.60, 31.20, 62.50 mg kg^{-1}	↑ SOD, PRX and GR (all doses) and CAT (0.48 to 31.20 mg. kg^{-1}).	Radetski et al. (2000)
Fabaceae	Hoagland solution	MTEs	*Pisum sativum*	CAT, APX, SOD, GR, GST, GPOX (leaves, roots)	Enzyme activity, Lipid peroxidation, Total glutathione	Cd	1, 3, 5, 7 d	0, 4, 40 μM	**Leaves:**↑SOD (1, 3 d, all doses; 5 d, 40 μM; 7 d, 4 μM), APX (all times; all doses), CAT (1, 3, 5 d, all doses; 7 d, 4 μM), GR (1 d, 40 μM; 3, 5, 7 d, 4 μM) and GST (all times, 40 μM). **Roots:**↑APX (all times, 4 μM; 3, 5, 7 d, 40 μM),	Dixit et al. (2001)

Table2 (*continued*)

Family	Medium	Nature of pollutant	Species	Oxidative stress indicator (organ)	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
									CAT (1 d, 4 μM), GR (1, 5, 7 d, all doses; 3 d, 40 μM) and GST (1, 5 d, all doses; 3, 7 d, 40 μM).↓SOD (1 d, 40 μM; 3, 5, 7 d, all doses), GPOX (1, 5, 7 d, 4 μM; 3 d, all doses) and CAT (5, 7 d, 40 μM).	
Fabaceae	Hoagland solution	MTEs	*Pisum sativum*	SOD, GR, CAT, GPOX (roots)	Enzyme activity, Total ascorbate, Total glutathione	Cd	14 d	50 μM	↑ SOD.↓GR and GPX.	Rodriguez-Serrano et al. (2006)
Fabaceae	Hoagland solution	MTEs	*Medicago sativa*	APX, CAT, PRX, SOD, GR (leaves)	Enzyme activity, Total glutathione	Se	1 week	0, 10, 20 μM	↓ APX and CAT (10, 20 μM) and SOD (20 μM).	Hajiboland and Amjad (2007)
Fabaceae	Hoagland solution	MTEs	*Vicia faba*	SOD, PRX, CAT (Leaves)	Enzyme activity, Lipid peroxidation, Comet assay	Cd	4 d	0, 5, 10 mg L^{-1}	↑ SOD (5 μM).↓PRX and CAT (5, 10 μM).	Lin et al. (2007)
Fabaceae	Hoagland solution	MTEs	*Glycine max*	SOD, GPOX (Leaves)	Enzyme activity, Lipid peroxidation, Total glutathione	Cd	48 h	200 μM	↓ SOD and GPOX.	Noriega et al. (2007)
Fabaceae	Hoagland solution	MTEs	*Medicago sativa*	GPOX, SOD, GR, APX (Roots)	Enzyme activity, Lipid peroxidation, Total ascorbate, Total glutathione	Hg	**(1)** 24 h **(2)** 0, 6, 12, 24, 48, 72 h	**(1)** 0, 1, 5, 10, 20, 40 μM **(2)** 0, 20 μM	**(1):**↑SOD (1, 5 μM), GPOX (20, 40 μM), GR (1μM) and APX (40 μM).↓SOD and GR (10, 20, 40 μM) and GPOX (1, 5 μM). **(2):**↑SOD (all times), GPOX (12, 24, 48, 72 h) and APX (48, 72 h) .↓GR (all times).	Zhou et al. (2007)
Fabaceae	Hoagland solution	MTEs	*Medicago truncatula*	PRX, GR, GST (roots)	Gene expression	Al	12 h	0, 25 μM	↑ *MtPER52* (x 13.30), *MtGR1* (× 2.7) and *MtGSTF2* (× 2.2).	Chandran et al. (2008)
Fabaceae	Hoagland solution	MTEs	*Medicago sativa*	NADH oxidase, SOD, CAT, APX, GR, GPOX (Leaves)	Enzyme activity, Lipid peroxidation, Total ascorbate, Total glutathione	Hg	**(1)** 7 d **(2)** 0, 2, 4, 6, 8, 10 d	**(1)** 0, 1, 5, 10, 20, 40 μM **(2)** 0, 20 μM	**(1):**↑NADHox, SOD, CAT and GR (20, 40 μM) and APX and GPOX (10, 20, 40 μM). **(2):**↑NADHox and CAT (6, 8, 10 d), SOD and GR (8, 10 d), APX (4, 6, 8 d) and GPOX (all times).	Zhou et al. (2008)
Fabaceae	Hoagland solution	MTEs	*Medicago sativa*	GR, APX, PRX, SOD (Leaves, Roots)	Enzyme activity, Total ascorbate, Total glutathione	Hg, Cd	7 d	0, 3, 10, 30 μM	**Leaves:** ↑PRX (Cd, 10, 30 μM; Hg, 30 μM) and GR (↑ with↑[Cd] and [Hg]). **Roots:**↑APX (Cd, Hg, all doses), PRX (Cd, 10, 30 μM; Hg, 30 μM) and GR (Cd).↓GR (Hg).	Sobrino-Plata et al. (2009)
Fabaceae	Hoagland solution	MTEs	*Trifolium repens*	CAT, SOD, GPX, APX (leaves, roots)	Enzyme activity, Lipid peroxidation	Cd	2 weeks	0, 400, 600 μM	**Leaves:**↑CAT, SOD, GPX and APX (400, 600 μM). **Roots:** no significant variation.	Wang and Song (2009)
Fabaceae	Hoagland solution	MTEs	*Medicago sativa*	NADH oxidase, SOD, APX, GR, GPOX (roots)	Enzyme activity, Lipid peroxidation, Total ascorbate, Total glutathione, Proline content	Hg	24 h	10 μM	↑NADHo × , SOD and GPOX. ↓GR and APX.	Zhou et al. (2009)
Fabaceae	Hoagland solution	MTEs	*Trifolium repens*	GPOX, NADH peroxidase (leaves)	Enzyme activity	Mn	9 d	100 μM	↓ NADHpr × (5, 9 h).	Dorling et al. (2011)
Fabaceae	Hoagland solution	MTEs	*Lotus corniculatus*	***SOD***, ***APX***, GPX, ***(M)*** ***DHAR***, ***GR*** (Roots)	***Enzyme activity***, Gene expression, Lipid peroxidation, Total ascorbate, Total glutathione	Al	14 d	0, 10, 20 μM	↑ FeSOD (both doses).↓CuZnSOD and DHAR (both doses).↑*LcFSDc* (10, 20 μM, × 2.20, × 4).↓*LcFSDp* (10, 20 μM, × 2.50, × 2.44) and *LcCSDc* (× 2.5), *LcGPX1* (× 2.5), *LcGPX4* (× 6.67), *LcDRp* (x 5) and *LcDRc* (× 6.67) (20 μM).	Navascués et al. (2012)
Fabaceae	Other medium	MTEs	*Trifolium alexandrinum*, *Medicago sativa*	CAT, APX, PRX (seedlings)	Enzyme activity, Proline content	Al	3, 7 d	0, 0.5, 1, 2, 4, 6 mM	***T. alexandrinum*:**↑APX (3, 7 d, all doses), PRX (3, 7 d, > 1 mM) and CAT (3 d, all doses; 7 d, > 1 mM). ***M. sativa*:**↑APX (3, 7 d, all doses), PRX (3 d, 2, 4, 6 mM; 7 d, 4, 6 mM) and CAT (3 d, 4, 6 mM; 7 d, all doses).↓CAT (3 d, 1, 2 mM).	Faheed (2008)

Table2 (*continued*)

Family	Medium	Nature of pollutant	Species	Oxidative stress indicator (organ)	Type of test	Stressor	Exposure time	Contamination level	Results	Reference
Fabaceae	Other medium	MTEs	*Pisum sativum*	***CAT***, ***APX***, ***SOD***, ***GR***, ***GPOX***, GST (leaves, roots)	***Enzyme activity***, Gene expression, Proline content	Al	24, 48 h	0, 10, 50 μM	**Leaves:**↑GPOX (48 h, 50 μM).↓APX and CAT (24 h, 10 μM; 48 h, 10, 50 μM), GR (24, 48 h, 10, 50 μM) and SOD (24, 48 h, 10 μM).↑*GST* (48 h, 10 μM, ×4), *CSD* (24 h, 10, 50 μM, ×5, ×7) and *APX* (24h, 10, 50 μM, ×6, ×3).↓*CSD* (48h, 50 μM, ×3), *MSD* (48 h, 10, 50 μM, ×6.25, ×12.5) and *CAT* (48 h, 10, 50 μM, ×2.94, ×33.33). **Roots:**↑GR (48 h, 50 μM).↓APX (24, 48 h, 10 μM), CAT and GPOX (48 h, 10 μM), GR (24 h, 10, 50 μM; 48 h, 10 μM) and SOD (48 h, 10, 50 μM). ↑*GST* (×5.2, ×3.7) and *CAT* (×2, ×3) (24, 48 h, 50 μM), *CSD* (×15) and *MSD* (×2.5) (24 h, 50 μM), *FSD* (48 h, 10, 50 μM, ×2, ×5) and *APX* (48 h, 50 μM, ×14).↓*CSD* (48 h, 10, 50 μM, ×1.79, ×2.27).	Panda and Matsumoto (2010)
Fabaceae	Other medium	MTEs	*Glycyrrhiza uralensis*	SOD, PRX, CAT (Cotyledons, Hypocotyls, Radicles)	Enzyme activity	Cd	7 d	0.05, 0.1, 0.2, 0.4 mmol	**Cotyledons/Radicles:** ↑SOD, PRX and CAT (all doses). **Hypocotyls:**↑PRX and CAT (all doses) and SOD (0.05, 0.1, 0.2 mmol).	Zheng et al. (2010)
Fabaceae	Other medium	Aromatic compounds	*Phaseolus vulgaris*	GST, GPX, GR (seeds)	Enzyme activity	PAHs	96 h	0, 0.02, 0.2, 2, 20, 200 μM	↑GST, GPX and GR .	Paskova et al. (2006)
Fabaceae	Other medium	Pesticides	*Phaseolus aureus*	SOD, GR, CAT, APX, GPOX (Leaves, Roots)	Enzyme activity, Lipid peroxidation, Proline content	BOA	7 d	0, 0.5, 1, 2.5, 5 mM	**Leaves:**↑SOD and APX (all doses) and CAT, GPOX and GR (>1 mM). **Roots:**↑SOD, CAT and GR (>1 mM) and APX and GPOX (all doses).	Batish et al. (2006)
Fabaceae	Soil	MTEs	*Trifolium pratense*	PRX, SOD (Leaves)	Enzyme activity, Total glutathione	As	10 weeks	0, 5, 10, 50 mg kg^{-1}	↑ SOD (10 mg.kg^{-1}) and PRX (all doses).↓SOD (5 mg kg^{-1}).	Mascher et al. (2002)
Fabaceae	Soil	MTEs	*Trifolium repens*	SOD (leaves, roots)	Enzyme activity, Lipid peroxidation	Cd, Pb, Zn	6 months	Ref: 0.59, 34.55, 61.60 mg kg^{-1} respectively; Poll.: 25.86, 1222, 1301 mg kg^{-1} respectively	**Leaves:**↓SOD. **Roots:**↑SOD.	Bidar et al. (2007)
Fabaceae	Soil	MTEs	*Alhargi camelthorn*	CAT, GPX, SOD (leaves, stems, roots)	Enzyme activity, lipid peroxidation	Mn	in situ sampling	127.22 mg kg^{-1}	**Leaves**:↑CAT and SOD. Stems/Roots: no significant variations.	Boojar and Goodarzi (2008)
Fabaceae	Soil	MTEs	*Trifolium repens*	SOD (Leaves, Roots)	Enzyme activity, Lipid peroxidation, ELISA	Cd, Pb, Zn	6 months	S1: 14.5, 952, 1215 mg. kg^{-1} respectively; S2: 14.9, 945, 1191 mg.kg^{-1} respectively; S3: 13.9, 866, 1131 mg kg^{-1}	Leaves:↓with↑[MTEs]. Roots:↑with↑[MTEs].	Bidar et al. (2009)
Fabaceae	Soil	MTEs+organic compounds	*Medicago sativa*	PRX, APX, (M) DHAR, GR, SOD (leaves)	Enzyme activity, lipid peroxidation, total ascorbate, Total glutathione	Oil refiney sludges	9 w	Hydrocarbons: 336 g kg^{-1}; Cd: <2.5; Pb: 15.5; Zn: 150.6 (mg kg^{-1})	↑ SOD, PRX, (M)DHAR, GR	Martí et al. (2009)
Fabaceae	Soil	MTEs+organic compounds	*Trifolium repens*	APX, GPOX (leaves, roots)	Enzyme activity, Comet assay	Landfill leachates	10 weeks	Cd: 0.96; Pb: 12.30; Zn: 3.11; Cr: 647; PAHs: 5.17 (μg L^{-1})	**Leaves:** no significant variation. **Roots:**↑APX (diluted leachate (50%), pure leachate (100%)).	Manier et al. (2012)

Abbreviations: APX: ascorbate peroxidase, GR: glutathione reductase, DHAR: dehydroascorbate reductase, MDHAR: monodehydroascorbate reductase, SOD: superoxide dismutase, CAT: catalase, GPOX: guaiacol peroxidase, GST: glutathione transferase, GPX: glutathione peroxidase, PCS: phytochelatin synthase, PRX: peroxidase, MT: metallothionein, NADH: nicotinamide dehydrogenase, AOX: alternative oxidase, GLR: glutaredoxin, PrxR: peroxiredoxin, Trx: thioredoxin, CDNB: 1-chloro-2,4-dinitrobenzene, TCA: trichloroacetate, BOA: 2-benzoxazolinone, MTEs: metal trace elements, PAHs: polycyclic aromatic hydrocarbons.

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solutions (Cd, Cu, Cd+Cu) during 24 h. GPOX, APX, SOD, GR and CAT activities were measured in leaves and roots. Results for enzyme activities in leaves were similar to those obtained by Semane et al. (2007) except SOD activities which showed no significant variation. Exposure to Cu alone did not disturb enzyme activities in leaves excepted APX activities which increased during Cd+Cu exposure. In roots, GPOX activities increased during Cd and Cd+Cu exposures whereas GR one decreased in the same conditions. In *A. thaliana* exposed to Cu, APX and CAT activities decreased.

In 2011, Cuypers et al. performed an analogous work. *A. thaliana* 3-week old plants were exposed to Cd or Cu solutions during 24 h. APX, SOD, GR and CAT activities were measured in leaves and roots. Results obtained for APX and SOD activities in both organs are in accordance with those obtained by Smeets et al. (2009). Otherwise, CAT activities increased in leaves during Cd5 and Cu5 exposures whereas they decreased in roots during Cu5 exposure. GR activities increased in leaves during Cu5 exposure and in roots during Cd5 exposure.

The same year, Zaimoglu et al. (2011) exposed *B. oleracea* and *B. juncea* 6-week old plants to Cr solutions during 10 weeks. CAT, APX and GR activities were measured in leaf tissues. Both species showed a similar trend in response to Cr treatments, the total enzyme activities were higher in *B. oleracea* than in *B. juncea*. Cr treatments significantly decreased CAT activities depending on Cr concentrations in both species. A coordinated increase in APX and GR activities in both species under Cr stress played a role as signals to protect plants from Cr-induced stress. Results concerning APX and GR activities in *B. juncea* are in accordance with those obtained by Pandey et al. (2005). Briefly, authors exposed *B. juncea* 15-day old plants to Cr solutions during 15 days. SOD, APX, CAT, GR and GST activities were measured in leaves and in roots. Contrary to Zaimoglu et al. (2011) who showed a decrease of CAT activities in leaves of plants exposed to Cr, CAT activities increased whatever Cr concentrations. In roots, all enzyme activities increased but SOD and APX activities decreased for the strongest Cr concentration and the longest exposure time. According to Pandey et al. (2005), the results suggested that Cr induced depression in plant growth of *B. juncea* to be a function of increased cellular accumulation of Cr despite increases of some antioxidant enzyme activities.

2.2.1.2. Gene expression. In 2007, Semane et al. performed also gene expression analysis of *A. thaliana* PCS (*AtPCS1*) and GR (*AtGR1*) in leaves (Semane et al., 2007). *AtPCS1* expression level increased significantly and *AtGR1* expression level decreased significantly with Cd doses. Data obtained suggests that the plants respond to Cd stress by the ascorbate-glutathione defence network at both transcriptional and enzymatic level. It appears that *Arabidopsis* plants exposed to 1 μM Cd were able to adopt a new metabolic equilibrium, allowing them to cope with this metal. However, when exposed to 10 μM Cd, loss of cellular redox homeostasis resulted in oxidative stress and toxicity.

In 2009, Smeets et al. performed gene expression analysis in *A. thaliana* leaves and roots (Smeets et al., 2009; part 2.2.1.1 of this review). *A. thaliana* SOD (*AtCSD1*, *AtCSD2*, *AtFSD1* and *AtMSD1*), CAT (*AtCAT1*), APX (*AtAPX1*) and GR (*AtGR1*) gene expressions were measured. Contrary to Semane et al. (2007) who showed that *AtGR1* was significantly down-regulated with Cd doses in leaves of *A. thaliana*, results obtained in this experiment showed an up-regulation of *AtGR1* in leaves of *A. thaliana* exposed to Cd, Cu and mixtures of Cd and Cu. In roots, this gene is up-regulated during Cd exposure and down-regulated during exposure to remaining conditions (Cu, Cd+Cu). Otherwise, *AtCSD1* and *AtMSD1* (Cu, Cd+Cu), *AtAPX1* (all conditions), *AtCAT1* and *AtFSD1* (Cd+Cu) were up-regulated in leaves whereas *AtCSD2* (Cd, Cd+Cu) and *AtFSD1* (Cu) were down-regulated. In roots, *AtFSD1*, *AtCAT1* and *AtAPX1* were up-regulated during Cd exposure whereas *AtCSD1*, *AtCSD2*, *AtFSD1*, *AtMSD1* and *AtAPX1* (Cu and Cd+Cu exposures) and *AtCAT1* (Cu exposure) were down-regulated. Results obtained for *AtFSD1* are in accordance with those obtained by Cuypers et al. (2011) and Opdenakker et al. (2012). Those obtained for *AtCAT1*, *AtAPX1* and *AtGR1* are in accordance with results found in Cuypers et al. (2011).

Vanhoudt et al. (2009) exposed *A. thaliana* 17-day old plants to 0, 0.1, 1, 10 and 100 μM uranium (U)-spiked solutions for 3 days. *A. thaliana* SOD (*AtCSD1*, *AtFSD1* and *AtMSD1*) and CAT (*AtCAT1*) gene expressions were measured in leaves and roots. The aim of the study was to analyze oxidative stress related response in *A. thaliana* after U exposure. The authors observed a decrease of *AtCSD1* expression in roots and *AtFSD1* expression in leaves with increasing U concentration whereas *AtCAT1* expression increased in leaves with increasing U concentrations except for 100 μM U. Results indicate that oxidative stress plays an important role in U toxicity but suggest that plant responses differ for leaves and roots. In 2010, the same authors went into depth with their research. Indeed, impact of an additional stress was assessed and *A. thaliana* were exposed to U either with gamma-rays (Vanhoudt et al., 2010a) or Cd (Vanhoudt et al., 2010b). Concerning Cd and U study, experimental scheme was the same as in Vanhoudt et al. (2009). *A. thaliana* seedlings were grown on hydroponics during 14 days and then exposed for 3 days to μM U in combination with 5 μM Cd. Gene expressions of A. thaliana CATs (*AtCAT1*, *AtCAT2* and *AtCAT3*), SODs (*AtCSD1*, *AtCSD2*, *AtCSD3*, *AtFSD1*, *AtFSD2*, *AtFSD3* and *AtMSD1*), APX (*AtAPX1*) and GR (*AtGR1*) were studied. Oxidative stress related responses are highly affected by Cd while U influence is more limited. While for roots, based on an increase in *AtFSD1* gene expression, oxidative stress was suggested to be superoxide induced, in leaves on the other hand, hydrogen peroxide related genes (e.g. *AtCAT1*, *AtCAT2*, *AtCAT3*) were mostly altered. Results obtained for *AtFSD1* and *AtCAT2* in both organs are in accordance with those obtained by Cuypers et al. (2011). In 2011, the same team performed additional studies (Vanhoudt et al., 2011a,b) and reached very similar conclusions.

2.2.2. Exposure to aromatic compounds

A total of four relevant references were selected for this topic (Table 2).

2.2.2.1. Enzyme activity. In 2012, Ahammed et al. sprayed five plant species among them *Brassica campestris* and *Brassica rapa* with a phenanthrene (PHE)-spiked solution during 2 weeks. CAT, SOD, GPOX, GR and APX activities were measured in leaves. In *B. campestris*, enzyme activities increased (all doses). In *B. rapa*, enzyme activities were relatively stable even decreasing when PHE concentration increased. Indeed, an increase in GPOX, CAT, and APX (all doses) and GR (300 μM) activities as well as a decrease in SOD (all doses) and GR (30 μM) activities could be noted. *B. rapa* seems to be the most PHE-tolerant species whereas *B. campestris* seems to be the least tolerant.

2.2.2.2. Gene expression. In 2010, Weisman et al. exposed *A. thaliana* to PHE during 21 days. Seeds were grown on square Petri dishes containing half-strength Murashige and Skoog (MS) medium. Microarray assays were made and GST (*AtGSTU24*, *AtGSTF2*, *AtGSTF3*, *AtGSTF6*, *AtGSTZ1* and *AtGSTF7*), CAT (*AtCAT1*, *AtCAT2* and *AtCAT3*), SOD (*AtCSD1*, *AtCSD2* and *AtFSD1*) and APX (*AtAPX4* and *AtTAPX*) gene expressions were studied. *AtGSTU24*, *AtGSTZ1*, *AtCSD1* and *AtCSD2* were up-regulated by PHE. Results for *AtGSTU24* are in accordance with those obtained by Skipsey et al. (2011) in *A. thaliana* exposed to 1-chloro-2,4-dinitrobenzene

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(CDNB) during 24 h. Additionally, the microarray probe that recognized *AtGSTF2* and *AtGSTF3* indicated a 3.7-fold increase of the transcripts on phenanthrene. Similarly, the probe that binds the GSTs *AtGSTF7* and *AtGSTF6* indicated 12-fold upregulation of these genes. CATs (*AtCAT1*, *AtCAT2* and *AtCAT3*), APXs (*AtAPX4* and *AtTAPX*) and *AtFSD1* were down-regulated on PHE. While oxidative stress was occurring under phenanthrene treatment, several antioxidant genes were downregulated. This scenario can occur when plants invoke a positive feedback loop that amplifies ROS to serve as signaling molecules.

2.2.3. Exposure to pesticides

A total of seven relevant references were selected for this topic (Table 2).

2.2.3.1. Enzyme activity. In 2000, Radetski et al. exposed three terrestrial species among them *B. campestris* cv. *chinensis* to different concentrations of herbicide TCA (sodium trichloroacetate) in a growth test according to guideline OECD # 208 (OECD, 1984b). SOD, PRX, CAT and GR activities were measured in whole plant. An increase in SOD, PRX and GR (all doses) and CAT (0.48–31.20 mg kg^{-1}) activities was noted. The increase in antioxidant enzyme activities observed in this study ensured the detoxification of increased levels of active oxygen species, and presumably prevented the plants from undergoing oxidative stress damage.

Drążkiewicz et al. (2003b) exposed *A. thaliana* 3-week old plants to colchicine and H_2O_2 solutions. SOD, CAT, GPOX and APX activities were measured in leaves. In the presence of colchicine, SOD activities increased, while CAT, APX and GPOX activities decreased. Inhibitory H_2O_2 effects on the activity of the enzymes were found. Colchicine pre-treatment resulted in an increase in CAT activity and a further increase in SOD activity in plants treated with H_2O_2.

2.2.3.2. Gene expression. In 2007, Laloi et al. exposed *A. thaliana* 3-week old plants to paraquat solutions during 21 days. Wild-type and thylakoidal ascorbate peroxidase-overexpressing plants were used. Seeds were grown on square Petri dishes containing MS medium and were sprayed either with a solution of 20 μM paraquat. Microarray assays were made and *A. thaliana* APX (a thylakoidal, *AtTAPX* and a cytosolic, *AtAPX1*) gene expressions were measured in leaves. The suppressive effect of *AtTAPX* on H_2O_2 concentrations within the transgenic line was tested by comparing transcript levels of four genes known to be specifically activated during paraquat or H_2O_2 treatment. In *AtTAPX*-overexpressing plants that were exposed to paraquat, transcript levels *AtAPX1* were 2.7-fold lower than in paraquat-treated wild-type plants. Results for *AtAPX1* are in accordance with those obtained by Bulgakov et al. (2012) in *A. thaliana* culture cells exposed to paraquat during 1 h. The chloroplast-specific H_2O_2 scavenger can reduce the endogenous H_2O_2 level in plastids of *AtTAPX*-overexpressing plants. The reduced activation of these marker genes further confirms their H_2O_2-specific responsiveness. Under no stress, the overexpression of *AtTAPX* did not affect plant fitness and only very poorly affected gene expression. The overexpression of *AtTAPX* altered the stress sensitivity of plants only under conditions that endorsed selectively the release of a particular ROS and led to a higher resistance to $O_2^{\bullet-}$/H_2O_2 in paraquat-treated wild-type plants.

The same year, Ramel et al. (2007) exposed *A. thaliana* plantlets to ATZ solutions (0 and 10 μM). Seeds were grown on square Petri dishes containing MS medium. After cultivation, plantlets were transferred to fresh medium in the absence or presence of 10 μM ATZ. Microarray assays were made and APX (*AtAPX1*), GST (*AtGSTU20* and *AtGSTF11*) and CAT (*AtCAT3*) gene expressions were measured in leaves. *AtAPX1*, *AtGSTU20* and *AtGSTF11* were significantly down-regulated whereas *AtCAT3* showed no variation during exposure to atrazine.

In 2011, Skipsey et al. exposed *A. thaliana* culture cells to fenclorim (herbicide) during 60 min. *Arabidopsis* Col-0 cultures were grown in the dark in MS medium and used 5 days after subculturing. Chemical treatments were prepared as 100 mM stocks in acetone and added to the medium as a 1:1000 dilution. Control treatments consisted of 0.1% v/v acetone. *A. thaliana* GST (*AtGSTU19*, *AtGSTU24*, *AtGSTF8* and *AtGSTL1*) gene expressions were measured. *AtGSTF8*, *AtGSTU19* and *AtGSTU24* were up-regulated whereas *AtGSTL1* showed no variation.

2.2.4. Exposure to mixtures containing MTEs and organic compounds

To our knowledge, no study about exposure to mixtures containing MTEs and organic compounds was performed in Brassicaceae at enzymatic and transcriptomic levels.

2.3. Fabaceae

T. repens belongs to Fabaceae family. A few studies concerning enzyme activity and gene expression have been performed with this species which is well-studied in agronomy (Harmoney et al., 2001; Sanderson et al., 2005, 2012; Deak et al., 2007; Forster et al., 2013). Thus, an expanded bibliography on the Fabaceae family has been made and is presented in this section. The literature review was carried out on the basis of keyword search in Scopus and PubMed using combinations of the followings: '*Trifolium repens** white clover*Fabaceae*enzyme activity*gene expression*xenobiotics*metals*organics*pesticides*aromatic compounds*' in Topics. Publications which appeared relevant for the review were sorted using titles, abstracts and full texts. Concerning gene expression studies, only those using real-time PCR were selected. This procedure allowed us to select a corpus of 24 references (Table 2).

2.3.1. Exposure to MTEs

A total of twenty relevant references were selected for this topic (Table 2).

2.3.1.1. Enzyme activity. In 2001, Dixit et al. exposed *Pisum sativum* (pea) 15-day old plants to Cd-contaminated solutions during 7 days. APX, CAT, SOD, GR, GST and GPOX activities were measured in leaves and roots. All enzyme activities increased in leaves excepted GPOX activities which showed no significant variation. In roots, CAT (4 μM) and APX, GR and GST (all doses) activities increased whereas those of CAT (40 μM) and SOD and GPOX (all doses) decreased. Results for CAT, SOD and APX activities in leaves are in accordance with those obtained by Wang and Song (2009) in *T. repens*. Briefly, they exposed 10-stolon seedlings to Cd-contaminated solutions during 2 weeks and CAT, SOD, GPX and APX activities were measured in roots and leaves. In the upper part of the plant, Cd^{2+} exposure led to a significant decrease in SOD, CAT and GPX activities and an increase in APX activities. In contrast, the roots showed an increase in activities of antioxidative enzymes under Cd^{2+} stress. Ca^{2+} application diminished the Cd^{2+} effect on activities of antioxidative enzymes in the upper part, even though it did not significantly affect these enzymes in roots. Possible mechanisms for the action of Ca^{2+} on Cd^{2+} stress were considered to reduce Cd^{2+} accumulation, alleviate lipid peroxidation and promote activities of antioxidative enzymes.

In 2007, Zhou et al. exposed *Medicago sativa* 4-day old seedlings to Hg-contaminated solutions (0–40 μM, 24 h and 20 μM, 6–72 h). The aim of this study was to achieve a better understanding of the biological mechanisms for Hg-induced oxidative stresses in plants and to develop an appropriate method that can

be used to estimate the degree of toxicity in Hg-contaminated soils. GPOX, SOD, GR and APX activities were measured in roots. GPOX (20, 40 μM), SOD (1, 5 μM), GR (1 μM) and APX (40 μM) increased but GPOX (1, 5 μM), SOD and GR (10, 20, 40 μM) activities decreased too. One year later, the authors performed a similar experiment in *M. sativa* leaves (Zhou et al., 2008). 4-day old seedlings were exposed to Hg-contaminated solutions (0–40 μM, 7 days and 20 μM, 2–10 days) and NADH oxidase, SOD, CAT, APX, GR and GPOX activities were assayed in leaves. All enzyme activities were increased for the strongest doses (20, 40 μM). Results for GR in roots and in leaves and APX in roots are in accordance with those obtained by Sobrino-Plata et al. (2009). Briefly, they exposed *M. sativa* 2-week old plants to solutions spiked with Cd or Hg during 7 days. GR, APX, PRX and SOD activities were assayed in roots and leaves. GR activities increased in leaves with increasing concentration in Cd and Hg. In roots, APX activities increased during Cd and Hg exposure whereas GR activities increased during Hg exposure but decreased during Cd exposure.

2.3.1.2. Gene expression. In 2010, Panda and Matsumoto exposed *P. sativum* 4-day old seedlings to Al-contaminated solutions during 48 h. Gene expressions of CAT (*PsCAT*), APX (*PsCAPX*), SODs (*PsMSD*, *PsFSD* and *PsCSD*) and GST (*PsGST*) were analyzed by real-time PCR in roots and leaves. In roots below 10 μM Al, *PsGST* was almost the same as in control but in 50 μM Al *PsGST* increased 5-fold after 24-h suggesting the effect of Al-stress, though the increase fell by 2-fold after 48 h. The root showed 2- and 3-fold increase in *PsCAT* expression after 24 h and 48 h respectively in 50 μM-Al which was negligible in shoot after 48 h of 50 μM-Al treatment, though after 24 h it showed 2-fold increase indicating the involvement of *PsCAT* in controlling damage caused by H_2O_2. In leaves, 10 μM-Al showed considerable increase in expression especially after 24 h compared with 50 μM Al. But in roots there was no significant change observed except in 50 μM after 48 h, which showed almost 3-fold increase in its expression. *PsCAPX* expression was increased in both the Al concentrations in shoot. In roots after 48 h the *PsCSD* showed decreased expression, whereas after 24 h 50 μM Al showed much higher expression. *PsFSD* showed gradual increase in its expression after 24 and 48 h of treatment. In case of *PsMSD* only 50 μM treatment showed elevated expression of the gene. After 24 h, at 10 and 50 μM Al, *PsCSD* and *PsMSD* expression increased whereas at only 10 μM *PsFSD* showed increased expression in shoot. But after 48 h except 10 μM of Al all the other treatments showed low *PsFSD* expression. The increase in these different types of SODs suggests their involvement in defense mechanism under oxidative stress generated in the cell under Al stress. Results for *PsGST* in roots are in accordance with those obtained by Chandran et al. (2008) in *Medicago truncatula* and those obtained for *PsCSD* in roots are in accordance with results of Navascués et al. (2012) in *Lotus corniculatus*.

2.3.2. Exposure to aromatic compounds

Only 1 relevant reference (Paskova et al., 2006) was selected for this topic (Table 2).

2.3.2.1. Enzyme activity. In 2006, Paskova et al. exposed *Phaseolus vulgaris* seeds to PAH-spiked solutions during 96 h. 10 PAHs were tested: phenanthrene, 1,10-Phenanthroline, 4,7-Phenanthroline, 1, 7-Phenanthroline, benzo[*h*]quinolone, phenanthridine, anthracene, acridine, fluorene and carbazole. GST, GPX and GR activities were measured in seeds. GST, GPX and GR increased during exposure to most of PAHs. GST show no significant variation during carbazole exposure, GPX during anthracene, acridine and fluorene exposures and GR during anthracene, acridine, fluorene and carbazole exposures.

2.3.2.2. Gene expression. To our knowledge, no study about exposure to aromatic compounds was performed in Fabaceae at transcriptomic levels.

2.3.3. Exposure to pesticides

Only 1 relevant reference (Batish et al., 2006) was selected for this topic (Table 2).

2.3.3.1. Enzyme activity. In 2006, Batish et al. grew during 7 days *Phaseolus aureus* seeds in Petri dishes containing a range of 2-Benzoxazolinone (BOA). The aim was to determine whether phytotoxicity of BOA is due to induction of oxidative stress caused by generation of reactive oxygen species (ROS) and the changes in levels of antioxidant enzymes induced in response to BOA. SOD, GR, CAT, APX and GPOX activities were measured in roots and leaves. In response to BOA, there was a significant increase in the activities of scavenging enzymes SOD, APX, GPOX, CAT, and GR in root and leaf tissue of mung bean. At 5 mM BOA, GR activity in roots showed a nearly 22-fold increase over that in control. The present study concludes that BOA induces oxidative stress in mung bean through generation of ROS and upregulation of activities of various scavenging enzymes.

2.3.3.2. Gene expression. To our knowledge, no study about exposure to pesticides was performed in Fabaceae at transcriptomic level.

2.3.4. Exposure to mixtures containing MTEs and organic compounds

A total of two relevant references were selected for this topic (Table 2).

2.3.4.1. Enzyme activity. In 2012, Manier et al. exposed in microcosms simultaneously two test species (*T. repens* and *E. fetida*) to a field-collected reference soil spiked with a landfill leachate (metal and organic compound mixture) during 10 weeks. GPOX and APX activities were assayed in roots and leaves. No significant variation in enzyme activities was noted in leaves. On the contrary, an increase in APX activities in roots was observed in plants exposed to pure leachate and 50% diluted leachate compared to those exposed to the reference soil. The response observed appears to be dose-dependent and linear in these experimental conditions. Results for APX activities in leaves are in accordance with those obtained by Martí et al. (2009) in *M. sativa* exposed to oil refinery sludges during 9 weeks.

2.3.4.2. Gene expression. To our knowledge, no study about exposure to mixtures containing MTEs and organic compounds was performed in Fabaceae at transcriptomic level.

3. Discussion

In our bibliography survey, 129 studies about oxidative stress in three model organisms exposed either to metal trace elements, aromatic compounds or pesticides were collected. Among references collected in this review, comparison between experiments is a challenge because matrix, xenobiotics, doses, exposure times and species are very different from a study to another. Indeed, results from these experiments are dependent from these factors and a change in one of them could make harder interpretation of results. First, a discussion about major parameters of experimental plans is done. Then, pollutants cited above are known to cause oxidative damages in organisms exposed. Consequently, similarities and

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differences in antioxidant systems in animals and plants are discussed in the second part. Finally, a comparison between gene expression and enzyme activity analysis is done.

3.1. Experimental design

3.1.1. Matrix

In animals like in plants, artificial (filter paper for animals, hydroponic cultures for plants and artificial soil for both) and natural media (spiked or polluted) were used in experimental plans. The contact (filter paper) and artificial soil tests are usually used for short-term studies (e.g. less than 4 days for contact test) designed to evaluate the impact of a specific chemical or substance on earthworm survival. One of the most used soils in ecotoxicology using earthworms is the OECD soil (OECD, 1984a). It is a sandy loam matrix which is built of quartz sand (70%), kaolinite clay (20%) and organic matter (sphagnum, 10%). Some work on the toxicity of metals to earthworms focused on the evaluation of concentration of metals in earthworm body and the soil (Gish and Christensen, 1973; Van Hook, 1974; Ireland, 1979; Ash and Lee, 1980). Such studies did not provide indication about the actual toxicity of the metals for worms, but give knowledge about concentrations of metals that adult worms can tolerate and/or accumulate. Therefore, testing procedures such as the earthworm contact and artificial soil test are also very useful indicators of acute toxicity (Neuhauser et al., 1985). Artificial soil test provides information regarding the concentrations of metals in soils that could actually cause the reduction or eradication of an earthworm population in soil ecosystems. Indeed, this soil is useful for relative toxicity assessment because it allows the worst-case situation, i.e. a maximal bioavailability of the metals tested. However, it may not be appropriate for ecotoxicological risk assessment since it may overestimate the toxicity of tested compounds (Ait Ali et al., 2004). It is recognized that each soil will have different chemical and physical parameters that affect the availability of metals. Artificial soil test allows only standardized testing.

In 1995, Spurgeon and Hopkin studied the effects of cadmium, copper, lead and zinc on *E. fetida* (Spurgeon and Hopkin, 1995). Results indicated that these metals are more toxic in OECD artificial soil than in field collected soils (Avonmouth, England, UK). For instance, calculation of the ratios of cocoon production allows to derive EC50s for zinc indicating that toxicity of this metal in OECD medium was 10 fold higher than that found in the field soil. OECD soil is a sandy loam matrix with few organic matters (10%) whereas natural soils are a mixture of minerals, organic matter, gases, liquids and an important amount of micro- and macro-organisms. Soil is a natural body that exists as part of the pedosphere and it performs four important functions: (1) medium for plant growth, (2) mean of water storage, supply and purification (3) modifier of the atmosphere and (4) habitat for organisms that take part in decomposition and creation of a habitat for other organisms. Soil consists of a solid phase (mineral and organic matters) as well as a porous phase that holds gases and water (Taylor and Ashcroft, 1972; Voroney, 2006). In artificial soil tests, metals are added to the soil as a solution of a soluble salt. Although a short period (up to 1 week) is allowed for stabilization, it is unlikely that sorption kinetics reach equilibrium within this time (van Wensem et al., 1994; Smit and van Gestel, 1996). Thus, worms may be exposed to higher concentrations of the selected metal species (such as perhaps the Zn^{2+} ion) than they would encounter in the field. Otherwise, metal uptake may be more closely related to total soil metal levels, while toxicity is determined by soluble concentrations. Thus in OECD and field soils with similar metal concentrations, accumulation may be comparable, while toxic effects may be more severe in OECD soil.

Terrestrial plants require soils not only as a source of nutrients but also as a means of anchorage of the entire plant by the root. In the mid of the 19th century, The German scientists Julius Sachs (1860) and Wilhelm Knop (1865) were able to demonstrate that higher plants can be cultivated in nutrient solutions without soil. This proved that plants are able to feed exclusively on inorganic materials without the need of soil or humates. From that time, the growth of plants in solution culture or hydroponics has been an important tool in the study of mineral nutrition such as the study of the uptake of plant nutrients, the antagonistic and synergistic behavior of plant nutrients, the effect of particular plant nutrients on growth and the synthesis of plant molecules of importance for crop quality. One of the best known nutrient solutions is that of Hoagland (Hoagland and Arnon, 1950).

There are major differences between hydroponic culture and culture of plants in soil. Nutrient solutions are not buffered, either in pH or in nutrient supply and plants grown in nutrient solutions are continuously and amply supplied with water. In order to overcome the problem of nutrient buffering, nutrient concentrations of solution cultures are generally much higher than in the soil solution (e.g. K^+, phosphate). However, a further problem of solution culture is that the composition of the nutrient solution changes with time of cultivation and for some nutrient concentrations may fall greatly whereas for others even toxicity levels may be reached. In order to overcome this problem, nutrient solutions should be changed frequently or the volume of solution per plant should be large (Parker and Norvell, 1999).

3.1.2. Pollutants

Xenobiotic are foreign chemical substances found within an organism that is not normally naturally produced by or expected to be present within that organism. In a context of environmental pollution, metal trace elements (MTEs), aromatic compounds and pesticides are xenobiotics. Each group of pollutant contains a huge variety of compounds. Indeed, MTEs include very toxic elements such as Cd, Pb or Hg, aromatic compounds are largely represented by polycyclic aromatic hydrocarbons (PAHs) and pesticides are mainly represented by herbicides and insecticides. Moreover, these pollutants compounds are known to cause severe, sometimes similar, damages to living organisms. For example, exposures to MTEs, aromatic compounds and pesticides can lead to oxidative stress in organisms (Drążkiewicz et al., 2003a; Lin et al., 2012a; Paskova et al., 2006).

Metal trace elements (MTEs) are natural constituents of the Earth's crust, but human activities (e.g. sludge or municipal compost, pesticides, emissions from municipal wastes incinerates, residues from metalliferous mines and smelting industries) have drastically altered their geochemical cycles and biochemical balance. MTEs are a member of an ill-defined subset of elements that exhibit metallic properties. These include the transition metals, some metalloids, lanthanides, and actinides. Any MTE species may be considered as "contaminant" if it occurs in a form or concentration that causes a detrimental human or environmental effect (e.g. Pb, Cd, Hg, As, Cr).

Some MTEs (e.g. Cd, Pb) have no known vital or beneficial effect on organisms, and their accumulation over time in the bodies of animals can cause serious illness. MTEs may disrupt metabolic functions in two ways: (1) they accumulate and thereby disrupt function in vital organs and glands such as the heart, brain, kidneys, bone, liver, etc.; (2) they remove the vital nutritional minerals from their original place, thereby, hindering their biological function. It is, however, impossible to live in an environment free of MTEs. In animals, MTEs can disturb reproduction, growth, survival, immune and nervous system. In plants, MTEs can disturb absorption and transport of essential elements, have an impact on growth and reproduction and affect photosystems (PSI and PSII).

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Metal ions are also well known inducers of oxidative stress. They can stimulate ROS production via two different mechanisms. The first one is related with the interference of metal-related processes and the second one with the generation of free radicals by ions with changeable valence. The second mode may also interfere with the first, but usually the main attention is paid to effects of metal ions with changeable valence.

One of the most studied aromatic compounds family is polycyclic aromatic hydrocarbons family (PAHs). It refers to a ubiquitous group of several hundred chemically-related, environmentally persistent organic compounds of various structures and varied toxicity. Most of them are formed by a process of thermal decomposition (pyrolysis) and subsequent recombination (pyrosynthesis) of organic molecules. PAHs enter the environment through various routes and are usually found as a mixture containing two or more of these compounds (e.g. soot). However, some PAHs are manufactured and these pure PAHs usually exist as colorless, white, or pale yellow solids. Polycyclic aromatic hydrocarbons affect organisms through various toxic actions. The mechanism of toxicity is considered to be interference with function of cellular membranes as well as with enzyme systems which are associated with the membrane. They have been shown to cause carcinogenic and mutagenic effects and are potent immunosuppressants. In animals, effects have been documented on immune system development, humoral immunity and on host resistence. Adverse effects on soil invertebrates include genotoxicity, adverse effects on reproduction, development, and immunity. In plants, PAH-induced phytotoxic effects are scarce. Some plants contain substances (e.g. phenols) that can protect against PAH effects whereas others can synthesize PAHs that act as growth hormones. The most extensively studied PAHs are the carcinogenics 7,12-dimethylbenzoanthracene (DMBA) and benzo(a)pyrene (BaP).

Pesticides are physical, chemical or biological agents intended to kill an undesirable plant and animal pests. Major classes of pesticides are: insecticides, herbicides and fungicides. It is important to note that most pesticides are synthetic agents, new to the environment and humans and, therefore, their effects on biological systems are poorly predictable. This group of toxicants may induce oxidative stress via several mechanisms: (i) being capable to enter redox cycles (reversible oxidation) accepting/donating electrons to cellular constituents, they may increase ROS level, (ii) at cellular metabolism some of pesticides may need involvement of reductants, such as glutathione, exhaust their reserves and result in decreased antioxidant potential, (iii) certain pesticides may inactivate antioxidant and associated enzymes leading to decreased antioxidant potential, (iv) interference with energy-providing processes may decrease supplement for metabolism and detoxification, and, finally, (v) modification of core vital processes, such as transcription and translation, in non-direct way may enhance steady-state ROS level.

3.1.3. Exposure time

Several times of exposure were used in references collected in this review. In order to better understand a physiological mechanism, it is advised to perform kinetic exposures rather than one or two periods of exposure. Indeed, measurements performed in organisms during one- or two-period exposures represent a given physiological state at a given time. Therefore, it does not inform on global physiological state of exposed organisms. On the other hand, kinetic exposures have at least three periods of exposure and one physiological state to each exposure time could be observed. Thus, by exploiting overall results, we could have an idea of global physiological state of exposed organisms. Among references collected in this review, 75% of studies performed exposures during at most two periods, short- or long-term exposures, whereas only 25% performed kinetic exposures (at least three periods).

3.2. Antioxidant systems

The antioxidant system in eukaryotes is made of both low and high molecular mass antioxidants. Low molecular mass antioxidant group includes water-soluble compounds such as reduced glutathione, ascorbic acid (vitamin C), and lipid-soluble ones such as carotenoids, retinol (vitamin A) and α-tocopherol (vitamin E). They usually operate as free radical scavengers. High molecular mass antioxidant group consists in specific or non-specific proteins. A specific group includes antioxidant enzymes such as superoxide dismutases (SODs), catalases (CATs), peroxidases (PRXs) which include glutathione peroxidases (GPXs) and ascorbate peroxidases (in plants, APXs), glutathione reductases (GRs). Non-specific high molecular mass antioxidants are represented by proteins that prevent ROS-induced damage by binding to transition metal ions such as metallothioneins (MTs) and phytochelatins (PCs) and by secondary defense enzymes such as glutathione transferases (GSTs).

In literature, the studies regarding impacts of oxidative stress mostly focused on enzyme activities variations. Experiments using gene expression analysis in order to explore oxidative stress mechanism are much less numerous and therefore sequence data are missing in databases. Even if sequence data are lacking in species of interest, it is actually still possible to measure gene expression levels. Indeed, many genes involved in important physiological functions are highly conserved between phyla. Phylogenetic conservation can thus be exploited to clone selected genes in species of interest. Therefore, nucleotidic sequences coding most proteins involved and/or related to oxidative stress were cloned in order to better understand how antioxidative mechanism works in our ecotoxicologically important test species (*E. fetida*, *B. oleracea* and *T. repens*).

Like for the literature based part of this review, an important data search in molecular databases was carried out first on the basis of keywords in Genbank™ (http://www.ncbi.nlm.nih.gov/genbank/) using combinations of the following keywords 'name of the species* (e.g. *E. fetida*) common name* (e.g. earthworms) species family* (e.g. Lumbricidae) name of enzyme or protein of interest* (e.g. catalase)' in Topics then by means of a BLAST when few sequences were available for a given candidate. This procedure was performed for each species. Then, by means of molecular biology techniques, we have been able to clone part of most missing targets.

Consensus-degenerated hybrid oligonucleotide primers (CODEHOPs) were used and degenerated primer design was conducted as described previously (Brulle et al., 2006). Thus, 6 candidates in *E. fetida*, 11 in *B. oleracea* and 10 in *T. repens* were obtained through this method (Tables S1, S2 and S3). This work would bring new molecular data which will be useful for us and others to explore oxidative stress mechanisms at transcriptional level in three promising models (Figure S1). However, cloning of some effectors may be an issue especially due to the lack of sequences available in databases. For instance, GSTs show relatively low-conserved areas when alignments were performed, consequently it was very difficult to design good quality degenerated primers for cloning. This could be explained by the fact that GST family is a diverse family of proteins that drive wide-ranging reactions. However, plants GSTs are easily categorized and well-conserved. The major problem comes from ancestral genetic multiplications driven to many independent genes which are very similar between each other. Sometimes, numerous conserved areas can be found in protein sequence alignments but cloning was still difficult. This is the case for gene encoding FeSOD in *T. repens*. Indeed, we were not able to clone this gene despite obvious observation of relatively conserved areas in protein sequences (data not shown). Absence of cloning might be due to the lack of expression in the studied conditions. According to Ferreira et al. (2002), which measured

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SODs (Cu/ZnSODs, FeSODs and MnSODs) activities, FeSODs activities are not often detected in plants.

Through literature review and cloning of candidates involved and/or related to oxidative stress, schematics of putative antioxidant systems in *E. fetida* (Fig. 1), *B. oleracea* (Fig. 2) and *T. repens* (Fig. 3) could be drawn. Fig. 1 has been done from literature data of the oxidative stress in animal cells (Matés et al., 1999; Kamata and Hirata, 1999; Brulle, 2005). Localization of major proteins involved in ROS scavenging presented in Fig. 1 is supposed to be similar in *E. fetida*. Figs. 2 and 3 have been adapted from Mittler et al. (2004) who proposed a general oxidative stress scheme in higher plants. Localization of major proteins involved in ROS scavenging presented in Figs. 2 and 3 are supposed to be similar in *B. oleracea* and *T. repens* respectively. For all figures, candidates in bold red are available in databanks and those in bold italic blue have been cloned.

As soon as cloning is completed, quantification of expression level of genes encoding antioxidative enzymes using real-time PCR is possible. However, cloning does not ensure quantification of expression level of a given target. Therefore, although quantifiable, gene expression level of some target genes may not display statistically significant variations in our experimental, making those genes not relevant for further analysis as biomarkers of exposure.

The superoxide dismutase enzymes (SODs) remove $O_2^{\bullet-}$ by catalyzing its dismutation, one $O_2^{\bullet-}$ being reduced to H_2O_2 and another oxidized to O_2 ($2O_2^{\bullet-}+2H^+ \rightarrow H_2O_2+O_2$). Animals have SODs containing active-site manganese (MnSOD) in the mitochondrial matrix and in peroxisomes (Fig. 1, top right and left respectively) as well as SODs with copper and zinc (CuZnSOD) in the rest of the cell (Fridovich, 1995) (Fig. 1, bottom right). Plants have more or less the same enzyme, namely MnSOD in mitochondrial matrix and in peroxisomes (Figs. 2 and 3, bottom right (MSD1) and middle right respectively) and CuZnSOD in chloroplast and cytosol (Figs. 2 and 3, top left (CSD2) and bottom left respectively) but some have iron-containing SODs (FeSOD) in the chloroplast (Alscher et al., 2002) (Fig. 2 (FSD3) and Fig. 3, top right). Whatever the metal, all SODs catalyze the above reaction (Fridovich, 1995; Halliwell and Gutteridge, 2006).

SODs work with superoxide reductases (SORs) to remove H_2O_2. CATs are not the most important in this context since there is little or no CAT in mitochondria and chloroplasts, where much $O_2^{\bullet-}$ is generated (Halliwell and Gutteridge, 2006). Most or all CATs in plants and animals is in peroxisomes (Fig. 1, top left; Figs. 2 and 3, middle right), to deal with H_2O_2 produced by oxidase enzymes (Schrader and Fahimi, 2004). Some CATs are located in cytosol (Brulle, 2005) (Fig. 1, bottom right).

Plants are rich in PRXs, enzymes that remove H_2O_2 by using it to oxidize a cosubstrate (Fig. 2, middle left (PRXCB); Fig. 3, bottom left (PRX22)). Many plant PRXs are non-specific, using multiple cosubstrates. APXs are located in most of cell compartments (Figs. 2 and 3). In plant chloroplast and cytosol, APXs can remove H_2O_2 by using vitamin C as a cosubstrate (Figs. 2 and 3, top left, top right and bottom left), oxidizing it to a poorly reactive ascorbyl free radical (Mano et al., 2001).

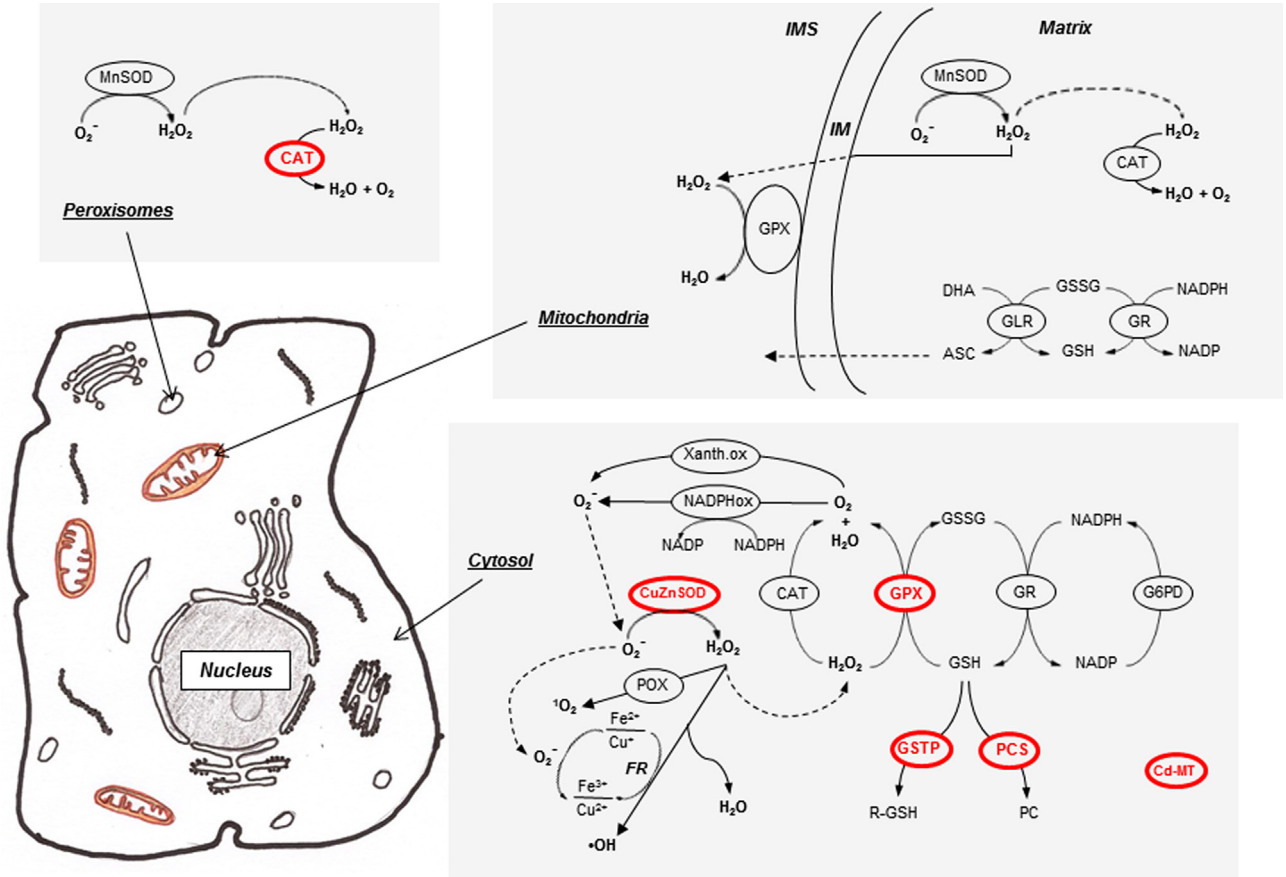

Fig. 1. (A) Localization of major proteins involved in ROS scavenging in *E. fetida*. Candidates in red are available in GenBank™. *Abbreviations*: $O_2^{\bullet-}$: superoxide radical, H_2O_2: hydrogen peroxide, •OH: hydroxyl radical, $^1O_2$: singlet oxygen, H_2O: water, O_2: dioxygen, MnSOD: manganese superoxide dismutase, CAT: catalase, GPX: glutathione peroxidase, DHA: dehydroascorbate, ASC: ascorbate, GLR: glutaredoxin, GSH: reduced glutathione, GSSG: oxidized glutathione, GR: glutathione reductase, NADP(H): nicotinamide adenine dinucleotide phosphate, IM: inner membrane, IMS: IM space, CuZnSOD: copper/zinc superoxide dismutase, G6PD: glucose-6-phosphate dehydrogenase, POX: peroxidase, FR: Fenton reaction, GSTP: pi glutathione transferase, PC: phytochelatin, PCS: PC synthase, Cd-MT: cadmium-metallothionein. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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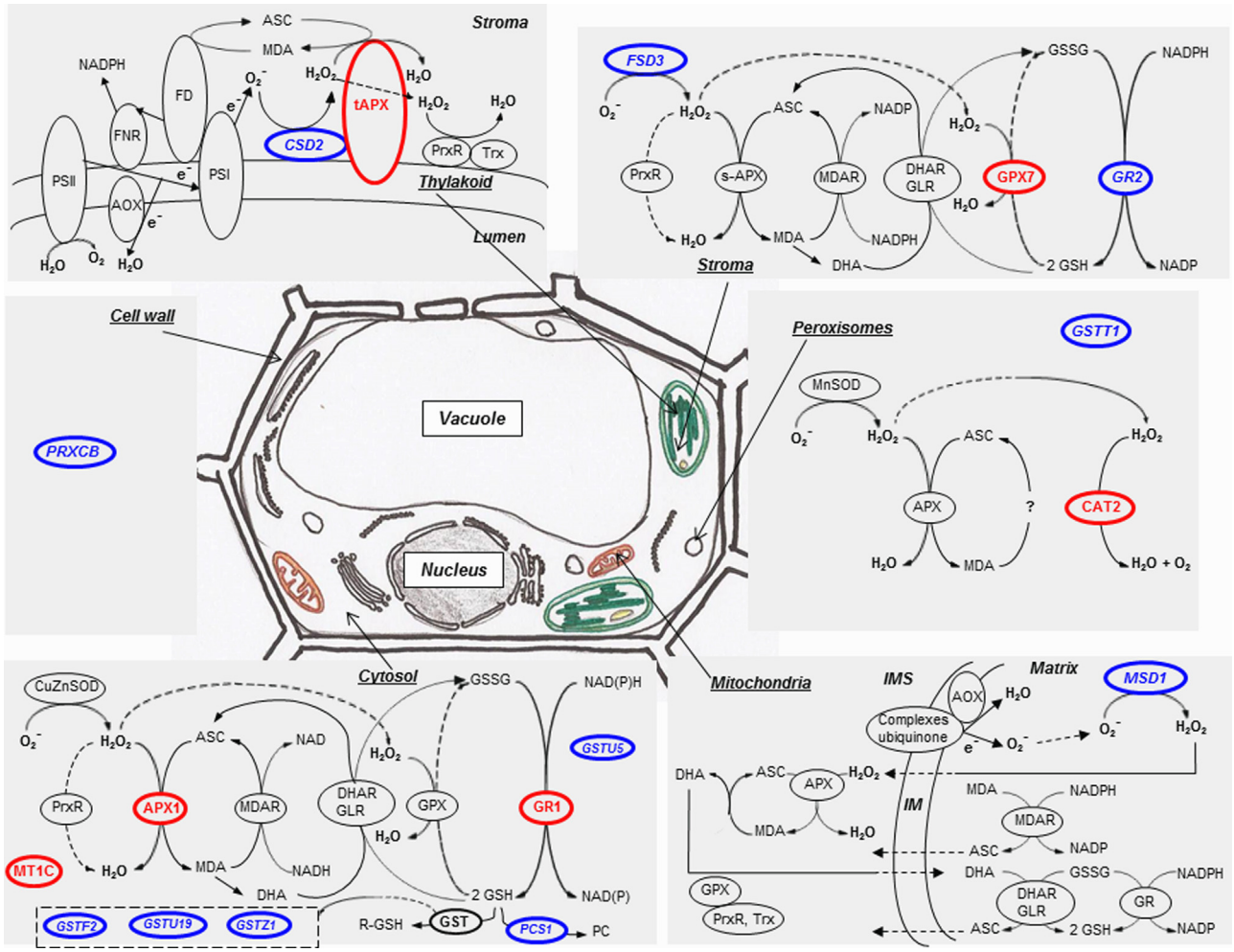

Fig. 2. (A) Localization of major proteins involved in ROS scavenging in *B. oleracea* (adapted from Mittler et al., 2004). Candidates in red are available in databanks (GenBank™, BolBASE), those in blue have been cloned. *Abbreviations*: PSI: photosystem I, PSII: photosystem II, AOX: amine oxidase, NADP(H): nicotinamide adenine dinucleotide phosphate, FD: ferredoxin, FNR: FD NADP reductase, ASC: ascorbate, DHA: dehydroascorbate, DHAR: DHA reductase, MDA: monodehydroascorbate, MDAR: MDA reductase, CSD2: copper/zinc superoxide dismutase 2, MSD1: manganese superoxide dismutase 1, FSD3: iron superoxide dismutase 3, tAPX: thylakoidal bound ascorbate peroxidase, s-APX: stromal ascorbate peroxidase, APX1: cytosolic ascorbate peroxidase, PrxR: peroxiredoxin, Trx: thioredoxin, GLR: glutaredoxin, H_2O: water, O_2: dioxygen, $O_2^{\bullet -}$: superoxide radical, H_2O_2: hydrogen peroxide, e^-: electron, GSH: reduced glutathione, GSSG: oxidized glutathione, GR1: cytosolic glutathione reductase, GR2: chloroplastic glutathione reductase, GPX7: glutathione peroxidase 7, GST: glutathione transferase, GSTF2: phi 2 GST, GSTU19: tau 19 GST, GSTU5: tau 5 GST, GSTT1: theta 1 GST, GSTZ1: zeta 1 GST, PRXCB: peroxidase CB, CAT2: catalase 2, IM: inner membrane, IMS: IM space, MT1C: metallothionein 1C, PC: phytochelatin, PCS1: PC synthase 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Until recently, it was thought that the most important H_2O_2-removing enzymes in animals are GPXs, a family of selenium-containing enzymes that remove H_2O_2 by coupling its reduction to water with oxidation of reduced glutathione (GSH), a thiol-containing tripeptide (glu-cys-gly) ($H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$; Brigelius-Flohe, 1999). The product, oxidized glutathione (GSSG), consists of two GSH linked by a disulfide bridge, and can be converted back to GSH by GR enzymes (Fig. 1, bottom right). On the other hand, selenium plays little role in plants. The chloroplast appears to lack selenoprotein GPX activity because they are rare in plants and APXs do some or all of the job of H_2O_2 removal (Mano et al., 2001). GPX-like activity has been identified in chloroplasts, mitochondria and cytoplasm in some plant species (Figs. 2 and 3, top right, bottom right and top left respectively) and genes similar to those encoding GPXs in animals have been identified in several plant genomes. Plant GPXs have Cys rather than seleno Cys at the active sites, which decreases their catalytic activity as compared with selenoprotein GPX enzymes. Indeed, at least some of the plant enzymes may prefer thioredoxins (Trxs) to GSH as a substrate (Herbette et al., 2002; Rodriguez Milla et al., 2003).

According to Rhee et al. (2005), peroxiredoxins (PrxRs) may be the most important H_2O_2-removal systems in eukaryotes and bacteria. They are homodimers and contain no prosthetic groups: The redox reactions are dependent on Cys at the active sites. PrxRs are slower at catalyzing H_2O_2 removal than GPXs but they can compensate for this through their presence in all subcellular organelles and in the cytosol (Figs. 2 and 3). PrxRs are readily inactivated by H_2O_2.

The secondary defense enzymes glutathione transferases (GSTs, EC 2.5.1.18) are a diverse family of proteins that share a similar three-dimensional structure and possess a well-defined glutathione-binding domain at their active sites (Armstrong, 1997). In eukaryotes, GSTs are divided into 14 distinct classes: Alpha, Beta, Delta, Epsilon, Mu, Nu, Pi, Omega, Phi, Tau, Zeta, Theta and Sigma GSTs are cytosolic and Kappa GSTs are mitochondrial. Alpha, Mu and Pi GSTs are specific to animals (Buetler and Eaton, 1992) (Fig. 1, bottom right) whereas Tau and Phi GSTs are plant-specific (Banerjee and Goswami, 2010) (Fig. 2, middle right and bottom left; Fig. 3, bottom left). Both plant-specific GSTs have major roles in herbicide detoxification (Marrs, 1996; Edwards et al., 2000) and can function as GPXs in order to counteract oxidative stress (Roxas

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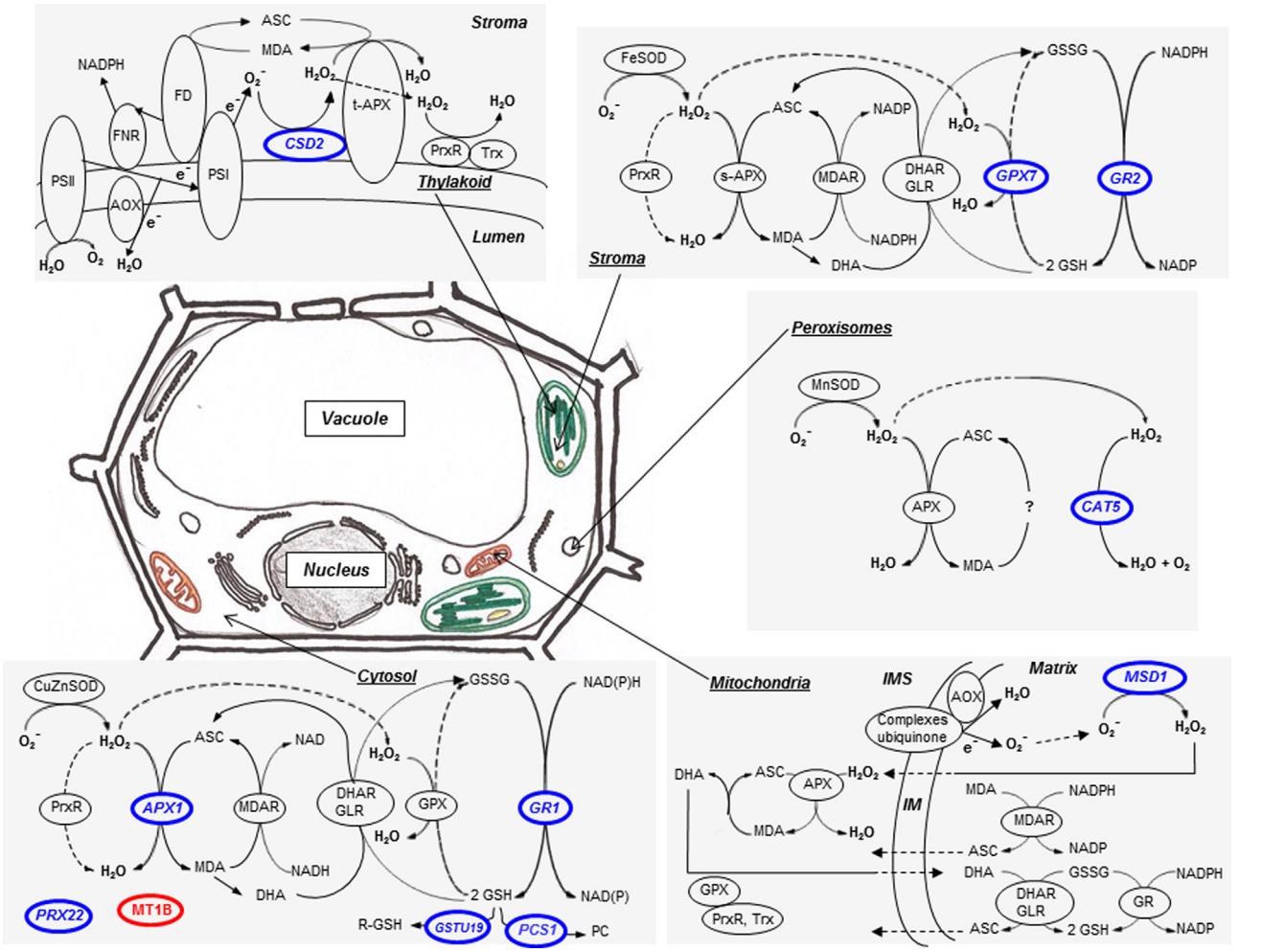

Fig. 3. (A) Localization of major proteins involved in ROS scavenging in *T. repens* (adpted from Mittler et al., 2004). Candidates in red are available in GenBank™, those in blue have been cloned. *Abbreviations:* PSI: photosystem I, PSII: photosystem II, AOX: amine oxidase, NADP(H): nicotinamide adenine dinucleotide phosphate, FD: ferredoxin, FNR: FD NADP reductase, ASC: ascorbate, DHA: dehydroascorbate, DHAR: DHA reductase, MDA: monodehydroascorbate, MDAR: MDA reductase, CSD2: copper/zinc superoxide dismutase 2, MSD1: manganese superoxide dismutase 1, FeSOD: iron superoxide dismutase, t-APX: thylakoidal bound ascorbate peroxidase, s-APX: stromal ascorbate peroxidase, APX1: cytosolic ascorbate peroxidase, PrxR: peroxiredoxin, Trx: thioredoxin, GLR: glutaredoxin, H_2O: water, O_2: dioxygen, $O_2^{\bullet-}$: superoxide radical, H_2O_2: hydrogen peroxide, e^-: electron, GSH: reduced glutathione, GSSG: oxidized glutathione, GR1: cytosolic glutathione reductase, GR2: chloroplastic glutathione reductase, GPX7: glutathione peroxidase 7, GSTU19: tau 19 glutathione transferase, PRX22: peroxidase 22, CAT5: catalase 5, IM: inner membrane, IMS: IM space, MT1B: metallothionein 1B, PC: phytochelatin, PCS1: PC synthase 1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

et al., 1997; Cummins et al., 1999). Theta and Zeta GSTs are both found in animals and in plants (Banerjee and Goswami, 2010).

Metallothioneins (MTs) are small cysteine rich proteins that range in size from 4 to 8 kDa and bind various metals like copper, cadmium, zinc, nickel, etc. (Hamer, 1986). MTs have been found in a wide variety of organisms including animals, cyanobacteria, fungi, and higher plants (Coyle et al., 2002; Cobbett and Goldsbrough, 2002). In animals, they are encoded by a gene and those which are inducible by a metallic stress are named type II MTs (Fig. 1, bottom right). They are mainly known for their roles in detoxification of MTEs such as Cd and in homeostasis of essential elements such as Zn (Palmiter, 1998; Klaassen et al.,1999). Plant MTs (Figs. 2 and 3, bottom left) have been sub-divided into three classes based on the arrangement of cysteine residues (Zhou et al., 2006). Most plant MTs belong to class I wherein the two cysteine rich domains are separated by a spacer of about 30–40 amino acids.

Phytochelatins (PCs) are peptides synthesized post-transcriptionally by constitutive phytochelatin synthase (PCS; Fig. 1, bottom right; Figs. 2 and 3, bottom left) (a.k.a γ-glutamylcysteine dipeptidyl transferase) using reduced glutathione as a substrate (Rea et al., 2004). PCS itself is rapidly induced by heavy metal ions such as As^-, Se^-, Ag^+, Cd^{2+}, Cu^{2+}, Hg^{2+} and Pb^{2+} (Grill et al.,1985). PCs possess the general structure (γ-Glu-Cys)*n*-Gly where '*n*' ranges from 2 to 11, although PC2 and PC3 appear to be the predominant forms (Raab et al., 2004). In plants, PCs are high-affinity chelators of metals and play major roles in the detoxification of Cd^{2+} and arsenate (Hall, 2002; Clemens, 2006). However, they appear to play no role in Ni and Zn detoxification whilst their role in Cu tolerance remains unresolved (Hall, 2002). PCS genes have been already found in animal species from eight phyla, including species that are widely used in environmental toxicity testing and environmental monitoring, such as the oyster *Crassostrea gigas* (Bundy et al., 2014), as well as nematodes (e.g. *Caenorhabditis elegans* (Hughes et al., 2009)) and earthworms (e.g. *E. fetida* (Brulle et al., 2008a)). However, presence of PCs was only observed in *C. elegans* (Hughes et al., 2009) and in *L. rubellus* (Liebeke et al., 2013).

Finally, there are sacrificial antioxidants, agents that are preferentially oxidized by reactive species to preserve more important biomolecules. For example, ascorbate can scavenge most ROS, including $O_2^{\bullet-}$, $OH^{\bullet}$, $RO_2^{\bullet}$, and ONOOH, as can GSH. Tocopherols are good scavengers of peroxyl radicals and help to protect membranes against lipid peroxidation by interrupting the chain reaction. Reaction is with the phenolic –OH group of the tocopherol structure (Halliwell and Gutteridge, 2006).

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Ascorbate and tocopherols form are poorly reactive radicals (Smirnoff, 2001; Halliwell and Gutteridge, 2006). Plants can make all the ascorbate and tocopherols they want contrary to humans who need to eat the plants to get them. Most animals can still make ascorbate, but the terminal step in its synthesis generates H_2O_2 (Puskas et al., 1998). Ascorbate synthesis in plants uses a different pathway that does not make H_2O_2 (Smirnoff, 2001).

3.3. Gene expression vs. enzyme activity

In our literature survey, most studies (72.9 percent) focused on levels of activity of oxidative stress defense enzymes as biomarkers of exposure to assess effects of stress induced by contaminants. As observed from those papers, most biochemical techniques allow measurement of total enzyme activity of a given protein family but do not allow detection of each isoform of this family. For instance, by using enzyme activity analysis, three classes of SODs have been well-studied: Cu/ZnSOD, MnSOD and FeSOD. The sensitivity of Cu/ZnSOD to cyanide is used as a diagnostic tool to distinguish Cu/ZnSOD from FeSOD and MnSOD that are unaffected by cyanide. FeSOD is irreversibly inactivated by H_2O_2, whereas MnSOD is resistant to both inhibitors (Martinez et al., 2001; Drążkiewicz et al., 2007). More than 57 percent of studies assessing levels of activity of defense enzyme as biomarker used at most three protein classes. Usually, a focus is made on a few proteins. Keeping in mind that more than 34 enzymes devoted to oxidative stress defense exist in normal cells, this make difficult interpretations about the whole oxidative stress mechanisms taking place in cells, especially during exposure to xenobiotics. Comparison between experiments used in all studies referenced in this review is a challenge because pollutants, doses, matrix, species and exposure periods are very different from a study to another. Nevertheless, as observed from literature, xenobiotic exposures lead to oxidative stress in most organisms and some effectors could allow assessment of such stress (e.g. expression level of *Cd-mt* in *E. fetida*). Gene expression quantification using real-time PCR is more than an alternative method to enzyme activity measurements.

Quantification of gene expressions using real-time PCR, which is much more specific than an enzyme activity measures, allows gene expression quantification of a given enzyme as soon as its nucleotidic sequence is well known. On the contrary, most enzyme activities assessment enable measurement of total enzyme activity of a given protein family, which is less accurate. For example, in *A. thaliana*, three Cu/ZnSOD genes (*ATCSD1*, *ATCSD2* and *ATCSD3*), three FeSOD genes (*ATFSD1*, *ATFSD2* and *ATFSD3*) and two MnSOD genes (*ATMSD1* and *ATMSD2*) have been reported (Kliebenstein et al., 1999; Pan and Chung, 2002). Moreover, molecular analysis (gene expression) is the first level of integration of environmental stressor and it is supposed to respond to stressors earlier than biochemical markers (Brulle et al., 2011).

Some authors analyzed effectors at those two levels of organization in order to explore oxidative stress in organisms exposed to xenobiotics. For example, Remans et al. (2012) grew hydroponically *A. thaliana* seedlings and exposed plants to Zn-contaminated solutions (0, 100, 250 and 500 μM) during 24 h. SOD activities and gene expression of *AtCSD1*, *AtCSD2*, *AtFSD1*, *AtFSD2* and *AtFSD3* in *A. thaliana* leaves were measured. SOD activities increased whereas gene expression of *AtCSD1*, *AtCSD2*, *AtFSD1*, *AtFSD2* and *AtFSD3* decreased in leaves. These results support the existence of Zn-specific signal transduction pathways influencing antioxidative responses.

Combination of biochemical and molecular techniques allowed authors to improve understanding of oxidative stress mechanism. For example, if gene expression of a target gene is up-regulated (or down-regulated) and its corresponding enzyme activity is increased (or decreased), one could conclude that candidate is regulated at transcriptional level. On the contrary, if gene expression of a target gene is up-regulated (or down-regulated) and its corresponding enzyme activity is decreased (or increased), we could conclude that candidate has a post-transcriptional regulation.

4. Conclusion

A literature review of the antioxidant responses of three terrestrial species living in close contact with soil have been done. It appears that many oxidative stress enzyme activities have already been measured in those species and in others but it remains especially difficult to distinguish the involvement of a specific enzyme when oxidative stress occurs. Gene expression studies have also been considered since gene expression would allow this distinction at the transcriptomic level. Thus, in addition to the literature review, a data search in molecular database was carried out on the basis of keywords in Scopus, PubMed and Genbank™ for each species. Molecular data regarding *E. fetida* were already available in databases, but a lack of data regarding oxidative stress related genes was observed for *T. repens* and *B. oleracea*. By exploiting the conservation observed between species and using molecular biology techniques, we partially cloned missing candidates involved in oxidative stress and in metal detoxification in *E. fetida*, *T. repens* and *B. oleracea*. Thus, it is now possible to assess oxidative stress response at the trancriptional level, one of the first level of integration of xenobiotics exposure, enabling identification and analysis of early biomarkers candidates. As demonstrated in this review, this kind of analysis is fully complementary to the post-traductional biochemical level. Simultaneous use of molecular and biochemical techniques should enhance the understanding of oxidative stress mechanism. Furthermore, as responses to oxidative stress may be different between species for the same protein family (e.g. metallothioneins), one may keep in mind that model species should be used in a complementary manner in order to better understand the effects of xenobiotics on exposed organisms and to measure a wider range of biological effects (i.e. biomarkers).

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2014.04.024.

References

Agarwal, S., 2007. Increased antioxidant activity in *Cassia* seedlings under UV-B radiation. Biol. Plant. 51, 157–160.

Ahammed, G.J., Wang, M.M., Zhou, Y.H., Xia, X.J., Mao, W.H., Shi, K., Yu, J.Q., 2012. The growth, photosynthesis and antioxidant defense responses of five vegetable crops to phenanthrene stress. Ecotoxicol. Environ. Saf. 80, 132–139.

Ait Ali, N., Ater, M., Sunahara, G.I., Robidoux, P.Y., 2004. Phytotoxicity and bioaccumulation of copper and chromium using barley (*Hordeum vulgare* L.) in spiked artificial and natural forest soils. Ecotoxicol. Environ. Saf. 57, 363–374.

Alloway, B.J., Ayres, D.C., 1997. Chemical Principles of Environmental Pollution. Chapman and Hall, London.

Alscher, R.G., Erturk, N., Heath, L.S., 2002. Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. J. Exp. Bot. 53, 1331–1341.

Anderson, R.S., Patel, K.M., Roesijadi, G., 1999. Oyster metallothionein as an oxyradical scavenger: implications for hemocyte defense responses. Dev. Comp. Immunol. 23, 443–449.

Anderson, T.D., Zhu, K.Y., 2004. Synergistic and antagonistic effects of atrazine on the toxicity of organophosphorodithioate and organophosphorothioate insecticides to *Chironomus tentans* (Diptera: Chironomidae). Pestic. Biochem. Physiol. 80, 54–64.

Antunes, S.C., Castro, B.B., Nunes, B., Pereira, R., Gonçalves, F., 2008. in situ bioassay with *Eisenia andrei* to assess soil toxicity in an abandoned uranium mine. Ecotoxicol. Environ. Saf. 71, 620–631.

Aravind, P., Prasad, M.N.V., 2003. Zinc alleviates cadmium-induced oxidative stress in *Ceratophyllum demersum* L.: a free floating freshwater macrophyte. Plant Physiol. Biochem. 41, 391–397.

Armstrong, R.N., 1997. Structure, catalytic mechanism, and evolution of the glutathione transferases. Chem. Res. Toxicol. 10, 2–18.

Arnold, R.E., Hodson, M.E., Black, S., Davies, N.A., 2003. The influence of mineral solubility and soil solution concentration on the toxicity of copper to *Eisenia fetida* Savigny. Pedobiologia 47, 622–632.

Arzate-Cárdenas, M.A., Martínez-Jerónimo, F., 2012. Energy reserve modification in different age groups of *Daphnia schoedleri* (Anomopoda: Daphniidae) exposed to hexavalent chromium. Environ. Toxicol. Pharmacol. 34, 106–116.

Ash, C.P.J., Lee, D.L., 1980. Lead, cadmium, copper and iron in earthworms from road side sites. Environ. Pollut. 22, 59–67.

Avsian-Kretchmer, O., Gueta-Dahan, Y., Lev-Yadun, S., Gollop, R., Ben-Hayyim, G., 2004. The salt-stress signal transduction pathway that activates the gpx1 promoter is mediated by intracellular H_2O_2, different from the pathway induced by extracellular H_2O_2. Plant Physiol. 135, 1685–1696.

Ayele, M., Haas, B.J., Kumar, N., Wu, H., Xiao, Y., Van Aken, S., Utterback, T.R., Wortman, J.R., White, O.R., Town, C.D., 2005. Whole genome shotgun sequencing of *Brassica oleracea* and its application to gene discovery and annotation in *Arabidopsis*. Genome Res. 15, 487–495.

Balestrasse, K.B., Gardey, L., Gallego, S.M., Tomaro, M.L., 2001. Response of antioxidant defence system in soybean nodules and roots subjected to cadmium stress. Aust. J. Plant Physiol. 28, 497–504.

Banerjee, S., Goswami, R., 2010. GST profile expression study in some selected plants: in silico approach. Mol. Cell. Biochem. 336, 109–126.

Bartha, B., Huber, C., Harpaintner, R., Schroder, P., 2010. Effects of acetaminophen in *Brassica juncea* L. Czern.: investigation of uptake, translocation, detoxification, and the induced defense pathways. Environ. Sci. Pollut. Res. 17, 1553–1562.

Batish, D.R., Singh, H.P., Setia, N., Kaur, S., Kohli, R.K., 2006. 2-Benzoxazolinone (BOA) induced oxidative stress, lipid peroxidation and changes in some antioxidant enzyme activities in mung bean (*Phaseolus aureus*). Plant Physiol. Biochem. 44, 819–827.

Bernard, F., Brulle, F., Douay, F., Lemière, S., Demuynck, S., Vandenbulcke, F., 2010. Metallic trace element body burdens and gene expression analysis of biomarker candidates in *Eisenia fetida*, using an "exposure/depuration" experimental scheme with field soils. Ecotoxicol. Environ. Saf. 73, 1034–1045.

Berthelot, Y., Valton, E., Auroy, A., Trottier, B., Robidoux, P.Y., 2008. Integration of toxicological and chemical tools to assess the bioavailability of metals and energetic compounds in contaminated soils. Chemosphere 74, 166–177.

Bhuiyan, M.S.U., Min, S.R., Jeong, W.J., Sultana, S., Choi, K.S., Lee, Y., Liu, J.R., 2011. Overexpression of *AtATM3* in *Brassica juncea* confers enhanced heavy metal tolerance and accumulation. Plant Cell Tissue Organ Cult. 107, 69–77.

Bidar, G., Garçon, G., Pruvot, C., Dewaele, D., Cazier, F., Douay, F., Shirali, P., 2007. Behavior of *Trifolium repens* and *Lolium perenne* growing in a heavy metal contaminated field: plant metal concentration and phytotoxicity. Environ. Pollut. 147, 546–553.

Bidar, G., Pruvot, C., Garçon, G., Verdin, A., Shirali, P., Douay, F., 2009. Seasonal and annual variations of metal uptake, bioaccumulation, and toxicity in *Trifolium repens* and *Lolium perenne* growing in a heavy metal-contaminated field. Environ. Sci. Pollut. Res. 16, 42–53.

Bigorgne, E., Foucaud, L., Lapied, E., Labille, J., Botta, C., Sirguey, C., Falla, J., Rose, J., Joner, E.J., Rodius, F., Nahmani, J., 2011. Ecotoxicological assessment of TiO_2 byproducts on the earthworm *Eisenia fetida*. Environ. Pollut. 159, 2698–2705.

Bigorgne, E., Foucaud, L., Caillet, C., Giambérini, L., Nahmani, J., Thomas, F., Rodius, F., 2012. Cellular and molecular responses of *E. fetida* coelomocytes exposed to TiO_2 nanoparticles. J. Nanopart. Res., 14.

Bolwell, G.P., Wojtaszek, P., 1997. Mechanisms for the generation of reactive oxygen species in plant defence – a broad perspective. Physiol. Mol. Plant Pathol. 51, 347–366.

Boojar, M.M.A., Goodarzi, F., 2008. Comparative evaluation of oxidative stress status and manganese availability in plants growing on manganese mine. Ecotoxicol. Environ. Saf. 71, 692–699.

Booth, L.H., O'Halloran, K., 2001. A comparison of biomarker responses in the earthworm *Aporrectodea caliginosa* to the organophosphorus insecticides diazinon and chlorpyrifos. Environ. Toxicol. Chem. 20 (11), 2494–2502.

Brigelius-Flohe, R., 1999. Tissue-specific functions of individual glutathione peroxidases. Free Radic. Biol. Med. 27, 951–965.

Brulle, F., 2005. Evaluation des risques engendrés par la présence de métaux polluants: identification de biomarqueurs potentiels chez des invertébrés. Master degree report. University of Lille, 1 pp.

Brulle, F., Mitta, G., Cocquerelle, C., Vieau, D., Lemière, S., Leprêtre, A., Vandenbulcke, F., 2006. Cloning and real-time PCR testing of 14 potential biomarkers in *Eisenia fetida* following cadmium exposure. Environ. Sci. Technol. 40, 2844–2850.

Brulle, F., Mitta, G., Leroux, R., Lemière, S., Leprêtre, A., Vandenbulcke, F., 2007. The strong induction of metallothionein gene following cadmium exposure transiently affects the expression of many genes in *Eisenia fetida*: a trade-off mechanism? Comp. Biochem. Physiol. – C Toxicol. Pharmacol. 144, 334–341.

Brulle, F., Cocquerelle, C., Mitta, G., Castric, V., Douay, F., Leprêtre, A., Vandenbulcke, F., 2008a. Identification and expression profile of gene transcripts differentially expressed during metallic exposure in *Eisenia fetida* coelomocytes. Dev. Comp. Immunol. 32, 1441–1453.

Brulle, F., Cocquerelle, C., Wamalah, A.N., Morgan, A.J., Kille, P., Leprêtre, A., Vandenbulcke, F., 2008b. cDNA cloning and expression analysis of *Eisenia fetida* (Annelida: Oligochaeta) phytochelatin synthase under cadmium exposure. Ecotoxicol. Environ. Saf. 71, 47–55.

Brulle, F., Lemière, S., Waterlot, C., Douay, F., Vandenbulcke, F., 2011. Gene expression analysis of 4 biomarker candidates in *Eisenia fetida* exposed to an environmental metallic trace elements gradient: a microcosm study. Sci. Total Environ. 409, 5470–5482.

Brunetti, P., Zanella, L., Proia, A., De Paolis, A., Falasca, G., Altamura, M.M., Sanità di Toppi, L., Costantino, P., Cardarelli, M., 2011. Cadmium tolerance and phytochelatin content of *Arabidopsis* seedlings over-expressing the phytochelatin synthase gene *AtPCS1*. J. Exp. Bot. 62, 5509–5519.

Buetler, T.M., Eaton, D.L., 1992. Glutathione S-transferases: amino acid sequence comparison, classification, and phylogenetic relationship. J. Environ. Sci. Health Part C Environ. Carcinogen. Ecotoxicol. Rev. 10, 181–203.

Bulgakov, V.P., Gorpenchenko, T.Y., Veremeichik, G.N., Shkryl, Y.N., Tchernoded, G.K., Bulgakov, D.V., Aminin, D.L., Zhuravlev, Y.N., 2012. The rolB gene suppresses reactive oxygen species in transformed plant cells through the sustained activation of antioxidant defense. Plant Physiol. 158, 1371–1381.

Bundy, J.G., Sidhu, J.K., Rana, F., Spurgeon, D.J., Svendsen, C., Wren, J.F., Stürzenbaum, S.R., Morgan AJand Kille, P., 2008. 'Systems toxicology' approach identifies coordinated metabolic responses to copper in a terrestrial non-model invertebrate, the earthworm *Lumbricus rubellus*. BMC Biol. 6, 25.

Bundy, J.G., Kille, P., Liebeke, M., Spurgeon, D.J., 2014. Metallothioneins may not be enough: the role of phytochelatins in invertebrate metal detoxification. Environ. Sci. Technol. 48, 885–886.

Chandran, D., Sharopova, N., Ivashuta, S., Gantt, J.S., VandenBosch, K.A., Samac, D.A., 2008. Transcriptome profiling identified novel genes associated with aluminum toxicity, resistance and tolerance in *Medicago truncatula*. Planta 228, 151–166.

Chen, C., Xue, S., Zhou, Q., Xie, X., 2011a. Multilevel ecotoxicity assessment of polycyclic musk in the earthworm *Eisenia fetida* using traditional and molecular endpoints. Ecotoxicology 20, 1949–1958.

Chen, C., Zhou, Q., Liu, S., Xiu, Z., 2011b. Acute toxicity, biochemical and gene expression responses of the earthworm *Eisenia fetida* exposed to polycyclic musks. Chemosphere 83, 1147–1154.

Chen, C., Zhou, Q.X., 2012. Lipid peroxidation and gene expression of antioxidant enzymes in response to polycyclic musks in earthworm Eisenia fetida. Huanjing Huaxue 33 (8), 2855–2861.

Chen, L., Guo, Y., Yang, L., Wang, Q., 2008. Synergistic defensive mechanism of phytochelatins and antioxidative enzymes in *Brassica chinensis* L. against Cd stress. Chin. Sci. Bull. 53, 1503–1511.

Chiang, H.C., Lo, J.C., Yeh, K.C., 2006. Genes associated with heavy metal tolerance and accumulation in Zn/Cd hyperaccumulator *Arabidopsis halleri*: a genomic survey with cDNA microarray. Environ. Sci. Technol. 40, 6792–6798.

Cho, U.H., Seo, N.H., 2005. Oxidative stress in *Arabidopsis thaliana* exposed to cadmium is due to hydrogen peroxide accumulation. Plant Sci. 168, 113–120.

Christensen, O., 1988. The direct effects of earthworms on nitrogen turnover in cultivated soils. Ecol. Bull. – Swed. Nat. Sci. Res. Council 39, 41–44.

Clemens, S., 2006. Evolution and function of phytochelatin synthases. J. Plant Physiol. 163, 319–332.

Cobbett, C., Goldsbrough, P., 2002. Phytochelatins and metallothioneins: roles in heavy metal detoxification and homeostasis. Annu. Rev. Plant Biol. 53, 159–182.

Colacevich, A., Sierra, M.J., Borghini, F., Millán, R., Sanchez-Hernández, J.C., 2011. Oxidative stress in earthworms short- and long-term exposed to highly Hg-contaminated soils. J. Hazard. Mater. 194, 135–143.

Corpas, F.J., Palma, J.M., Del Río, L.A., 1993. Evidence for the presence of proteolytic activity in peroxisomes. Eur. J. Cell Biol. 61, 81–85.

Cortet, J., Gomot-De Vauflery, A., Poinsot-Balaguer, N., Gomot, L., Texier, C., Cluzeau, D., 1999. The use of invertebrate soil fauna in monitoring pollutant effects. Eur. J. Soil Biol. 35, 115–134.

Coyle, P., Philcox, J.C., Carey, L.C., Rofe, A.M., 2002. Metallothionein: the multipurpose protein. Cell. Mol. Life Sci. 59, 627–647.

Cummins, I., Cole, D.J., Edwards, R., 1999. Plant J. 18, 285–292.

Cuypers, A., Karen, S., Jos, R., Kelly, O., Els, K., Tony, R., Nele, H., Nathalie, V., Suzy, V.S., Frank, V.B., Yves, G., Jan, C., Jaco, V., 2011. The cellular redox state as a modulator in cadmium and copper responses in *Arabidopsis thaliana* seedlings. J. Plant Physiol. 168, 309–316.

Deak, A., Hall, M.H., Sanderson, M.A., Archibald, D.D., 2007. Production and nutritive value of grazed simple and complex forage mixtures. Agron. J. 99, 814–821.

Del Río, L.A., Sandalio, L.M., Corpas, F.J., Palma, J.M., Barroso, J.B., 2006. Reactive oxygen species and reactive nitrogen species in peroxisomes. Production, scavenging, and role in cell signaling. Plant Physiol. 141, 330–335.

Dixit, V., Pandey, V., Shyam, R., 2001. Differential antioxidative responses to cadmium in roots and leaves of pea (*Pisum sativum* L. cv. Azad). J. Exp. Bot. 52, 1101–1109.

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Dixon, D.P., Skipsey, M., Edwards, R., 2010. Roles for glutathione transferases in plant secondary metabolism. Phytochemistry 71, 338–350.
Doke, N., 1983. Generation of superoxide anion by potato tuber protoplasts during the hypersensitive response to hyphal wall components of *Phytophthora infestans* and specific inhibition of the reaction by suppressors of hypersensitivity. Physiol. Plant Pathol. 23, 359–367.
Doke, N., Ohashi, Y., 1988. Involvement of an O_2 - generating system in the induction of necrotic lesions on tobacco leaves infected with tobacco mosaic virus. Physiol. Mol. Plant Pathol. 32, 163–175.
Dong, L., Gao, J., Xie, X., Zhou, Q., 2012. DNA damage and biochemical toxicity of antibiotics in soil on the earthworm *Eisenia fetida*. Chemosphere 89, 44–51.
Dorling, S.J., Leung, S., Anderson, C.W.N., Albert, N.W., McManus, M.T., 2011. Changes in 1-aminocyclopropane-1-carboxlate (ACC) oxidase expression and enzyme activity in response to excess manganese in white clover (*Trifolium repens* L.). Plant Physiol. Biochem. 49, 1013–1019.
Drążkiewicz, M., Skórzyńska-Polit, E., Krupa, Z., 2003a. Response of the ascorbate-glutathione cycle to excess copper in *Arabidopsis thaliana* (L.). Plant Sci. 164, 195–202.
Drążkiewicz, M., Skórzyńska-Polit, E., Wanke, M., Świezewska, E., 2003b. The activity of antioxidant enzymes in *Arabidopsis thaliana* exposed to colchicine and H_2O_2. Cell. Mol. Biol. Lett. 8, 777–781.
Drążkiewicz, M., Skórzyńska-Polit, E., Krupa, Z., 2004. Copper-induced oxidative stress and antioxidant defence in *Arabidopsis thaliana*. BioMetals 17, 379–387.
Drążkiewicz, M., Skórzyńska-Polit, E., Krupa, Z., 2007. The redox state and activity of superoxide dismutase classes in *Arabidopsis thaliana* under cadmium or copper stress. Chemosphere 67, 188–193.
Edwards, C.A., Lofty, J.R., 1977. Biology of Earthworms. 1977. Chapman and Hall, New York p. 333.
Edwards, C.A., 1988. Breakdown of animal, vegetable and industrial organic wastes by earthworm. In: Edwards, C.A., Neuhauser, E.F. (Eds.), Earthworms in Waste and Environmental Management, 1988. SPB, The Hague, pp. 21–31.
Edwards C.A., Bohlen P.J.,1996. Biology and ecology of earthworms. Third ed. Chapman and Hall, New York, p. 426.
Edwards, C.A., 1998. The use of earthworms in the breakdown and management of organic wastes. In: Edwards, C.A. (Ed.), Earthworm Ecology. CRC Press, Boca Raton, FL, USA, pp. 327–354.
Edwards, R., Dixon, D.P., Walbot, V., 2000. Plant glutathione S-transferases: enzymes with multiple functions in sickness and in health. Trends Plant Sci. 5, 193–198.
Eyidogan, F., Öz, M.T., 2007. Effect of salinity on antioxidant responses of chickpea seedlings. Acta Physiol. Plant. 29, 485–493.
Faheed, F.A., 2008. The effect of aluminium on oxidative stress-related enzyme activity in two clover species. Turk. J. Bot. 32, 345–352.
Ferreira, R.R., Fornazier, R.F., Vitória, A.P., Lea, P.J., Azevedo, R.A., 2002. Changes in antioxidant enzyme activities in soybean under cadmium stress. J. Plant Nutr. 25, 327–342.
Forster, J.W., Panter, S., Mouradov, A., Mason, J., Spangenberg, G.C., 2013. Transgenic technologies for enhanced molecular breeding of white clover (*Trifolium repens* L.). Crop Pasture Sci. 64, 26–38.
Foyer, C.H., Noctor, G., 2005. Redox homeostasis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. Plant Cell 17, 1866–1875.
Fridovich, I., 1995. Superoxide radical and SODs. Annu. Rev. Biochem. 64, 97–112.
Gaete, H., Hidalgo, M.E., Neaman, A., Ávila, G., 2010. Assessment of copper toxicity in soils using biomarkers of oxidative stress in *Eisenia foetida*. Evaluación de la toxicidad de cobre en suelos a través de biomarcadores de estrés oxidativo en *Eisenia foetida*. Quim. Nova 33, 566–570.
Gill, S.S., Tuteja, N., 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol. Biochem. 48, 909–930.
Gish, C.D., Christensen, R.E., 1973. Cadmium, nickel, lead and zinc in earthworms from roadside soil. Environ. Sci. Technol. 117, 1060–1062.
Gomes, T., Pereira, C.G., Cardoso, C., Pinheiro, J.P., Cancio, I., Bebianno, M.J., 2012. Accumulation and toxicity of copper oxide nanoparticles in the digestive gland of *Mytilus galloprovincialis*. Aquat. Toxicol. 118–119, 72–79.
Gong, P., Guan, X., Inouye, L.S., Pirooznia, M., Indest, K.J., Athow, R.S., Deng, Y., Perkins, E.J., 2007. Toxicogenomics analysis provides new insights into molecular mechanisms of the sublethal toxicity of 2,4,6-trinitrotoluene in *Eisenia fetida*. Environ. Sci. Technol. 41, 8195–8202.
Gong, P., Guan, X., Inouye, L.S., Deng, Y., Pirooznia, M., Perkins, E.J., 2008. Transcriptomic analysis of RDX and TNT interactive sublethal effects in the earthworm *Eisenia fetida*. BMC Genomics 9 (article no. S15).
Goupil, P., Souguir, D., Ferjani, E., Faure, O., Hitmi, A., Ledoigt, G., 2009. Expression of stress-related genes in tomato plants exposed to arsenic and chromium in nutrient solution. J. Plant Physiol. 166, 1446–1452.
Grill, E., Winnacker, E.L., Zenk, M.H., 1985. Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science 230, 674–676.
Hajiboland, R., Amjad, L., 2007. Does antioxidant capacity of leaves play a role in growth response to selenium at different sulfur nutritional status? Plant Soil Environ. 53, 207–215.
Hall, J.L., 2002. Cellular mechanisms for heavy metal detoxification and tolerance. J. Exp. Bot. 53, 1–11.
Halliwell, B., 1987. Oxidants and human disease: some new concepts. FASEB J. 1, 358–364.
Halliwell, B., Gutteridge, J.M.C., 2006. Free Radicals in Biology and Medicine, 4th ed. Clarendon Press, Oxford.
Hamer, D., 1986. Metallothionein. Annu. Rev. Biochem. 55, 913–951.
Harmoney, K.R., Moore, K.J., Brummer, E.C., Lee Burras, C., George, J.R., 2001. Spatial legume composition and diversity across seeded landscapes. Agron. J. 93, 992–1000.
Hasanuzzaman, M., Hossain, M.A., Fujita, M., 2012. Exogenous selenium pretreatment protects rapeseed seedlings from cadmium-induced oxidative stress by upregulating antioxidant defense and methylglyoxal detoxification systems. Biol. Trace Elem. Res. 149, 248–261.
Hayashi, Y., Heckmann, L.H., Simonsen, V., Scott-Fordsmand, J.J., 2013. Time-course profiling of molecular stress responses to silver nanoparticles in the earthworm *Eisenia fetida*. Ecotoxicol. Environ. Saf. 98, 219–226.
Herbette, S., Lenne, C., Leblanc, N., Julien, J.L., Drevet, J.R., Roeckel-Drevet, P., 2002. Two GPX-like proteins from *Lycopersicon esculentum* and *Helianthus annuus* are antioxidant enzymes with phospholipid hydroperoxide glutathione peroxidase and thioredoxin peroxidase activities. Eur. J. Biochem. 269, 2414–2420.
Hernández, J.A., Almansa, M.S., Del Río, L., Sevilla, F., 1993. Effect of salinity on metalloenzymes of oxygen metabolism in two leguminous plants. J. Plant Nutr. 16, 2539–2554.
Hsu, Y.T., Kao, C.H., 2004. Cadmium toxicity is reduced by nitric oxide in rice leaves. Plant Growth Regul. 42, 227–238.
Hoagland, R.R., Arnon, D.I., 1950. The water culture methods for growing plants without soil. California Agricultural Experiment Station Circular no. 347, pp. 1–32.
Hockner, M., Stefanon, K., Schuler, D., Fantur, R., de Vaufleury, A., Dallinger, R., 2009. Coping with cadmium exposure in various ways: the two helicid snails *Helix pomatia* and *Cantareus aspersus* share the metal transcription factor-2, but differ in promoter organization and transcription of their Cd-metallothionein genes. J. Exp. Zool. Part A: Ecol. Genet. Physiol. 311, 776–787.
Hughes, S.L., Bundy, J.G., Want, E.J., Kille, P., Sturzenbaum, S.R., 2009. The metabolomic responses of *Caenorhabditis elegans* to cadmium are largely independent of metallothionein status, but dominated by changes in cystathionine and phytochelatins. J. Proteome Res. 8, 3512–3519.
Iannelli, M.A., Pietrini, F., Fiore, L., Petrilli, L., Massacci, A., 2002. Antioxidant response to cadmium in *Phragmites australis* plants. Plant Physiol. Biochem. 40, 977–982.
Ireland, M.P., 1979. Metal accumulation by the earthworms *Lumbricus rubellus*, *Dendrobaena veneta*, and *Eiseniella tetradra* living in heavy metal polluted sites. Environ. Pollut. 13, 201–206.
Irizar, A., Izagirre, U., Diaz de Cerio, O., Marigómez, I., Soto, M., 2014. Zonation in the digestive tract of Eisenia fetida: implications in biomarker measurements for toxicity assessment. Comp. Biochem. Physiol. Part C 160, 42–53.
Kamata, H., Hirata, H., 1999. Redox regulation of cellular signalling. Cell. Signal. 11, 1–14.
Kautenburger, R., 2006. Genetic structure among earthworms (*Lumbricus terrestris* L.) from different sampling sites in western Germany based on random amplified polymorphic DNA. Pedobiologia 50, 257–266.
Kappus, H., 1987. A survey of chemicals inducing lipid peroxidation in biological systems. Chem. Phys. Lipids 45, 105–115.
Kaveh, R., Li, Y.S., Ranjbar, S., Tehrani, R., Brueck, C.L., Van Aken, B., 2013. Changes in *Arabidopsis thaliana* gene expression in response to silver nanoparticles and silver ions. Environ. Sci. Technol. 47, 10637–10644.
Khan, N.A., Singh, S., Nazar, R., 2007. Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (*Triticum aestivum*) cultivars differing in yield potential under cadmium stress. J. Agron. Crop Sci. 193, 435–444.
Klaassen, C.D., Liu, J., Choudhuri, S., 1999. Metallothionein: an intracellular protein to protect against cadmium toxicity. Annu. Rev. Pharmacol. Toxicol. 39, 267–294.
Kliebenstein, D.J., Dietrich, R.A., Martin, A.C., Last, R.L., Dangl, J.L., 1999. *LSD1* regulates salicylic acid induction of copper zinc superoxide dismutase in *Arabidopsis thaliana*. Mol. Plant–Microbe Interact. 12, 1022–1026.
Kumar, D., Yusuf, M.A., Singh, P., Sardar, M., Sarin, N.B., 2013. Modulation of antioxidant machinery in α-tocopherol-enriched transgenic *Brassica juncea* plants tolerant to abiotic stress conditions. Protoplasma 250, 1079–1089.
Kumari, M., Taylor, G.J., Deyholos, M.K., 2008. Transcriptomic responses to aluminum stress in roots of *Arabidopsis thaliana*. Mol. Genet. Genomics 279, 339–357.
Kusznierewicz, B., Baczek-Kwinta, R., Bartoszek, A., Piekarska, A., Huk, A., Manikowska, A., Antonkiewicz, J., Namieśnik, J., Konieczka, P., 2012. The dose-dependent influence of zinc and cadmium contamination of soil on their uptake and glucosinolate content in white cabbage (*Brassica oleracea* var. *capitata* f. alba). Environ. Toxicol. Chem. 31, 2482–2489.
Laloi, C., Stachowiak, M., Pers-Kamczyc, E., Warzych, E., Murgia, I., Apel, K., 2007. Cross-talk between singlet oxygen- and hydrogen peroxide-dependent signaling of stress responses in *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 104, 672–677.
Łaszczyca, P., Augustyniak, M., Babczyńska, A., Bednarska, K., Kafel, A., Migula, P., Wilczek, G., Witas, I., 2004. Profiles of enzymatic activity in earthworms from zinc, lead and cadmium polluted areas near Olkusz (Poland). Environ. Int. 30, 901–910.
Lavelle, P., Spain, A.V., 2001. Soil Ecology. Kluwer Academic Publishers, Dordrecht, Netherlands.
Leisinger, U., Rüfenacht, K., Fischer, B., Pesaro, M., Spengler, A., Zehnder, A.J.B., Eggen, R.I.L., 2001. The glutathione peroxidase homologous gene from *Chlamydomonas reinhardtii* is transcriptionally up-regulated by singlet oxygen. Plant Mol. Biol. 46, 395–408.
Lemiere, S., Cossu-Leguille, C., Charissou, A.M., Vasseur, P., 2005. DNA damage (comet assay) and 8-oxodGuo (HPLC-EC) in relation to oxidative stress in the freshwater bivalve *Unio tumidus*. Biomarkers 10, 41–57.

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Li, D., Hockaday, W.C., Masiello, C.A., Alvarez, P.J.J., 2011a. Earthworm avoidance of biochar can be mitigated by wetting. Soil Biol. Biochem. 43, 1732–1737.
Li, L.Z., Zhou, D.M., Peijnenburg, W.J.G.M., van Gestel, C.A.M., Jin, S.Y., Wang, Y.J., Wang, P., 2011b. Toxicity of zinc oxide nanoparticles in the earthworm, *Eisenia fetida* and subcellular fractionation of Zn. Environ. Int. 37, 1098–1104.
Li, W.J., Feng, H., Fan, J.H., Zhang, R.Q., Zhao, N.M., Liu, J.Y., 2000. Molecular cloning and expression of a phospholipid hydroperoxide glutathione peroxidase homolog in *Oryza sativa*. Biochim. Biophys. Acta – Gene Struct. Expr. 1493, 225–230.
Liang, S.H., Chen, S.C., Chen, C.Y., Kao, C.M., Yang, J.I., Shieh, B.S., Chen, J.H., Chen, C.C., 2011. Cadmium-induced earthworm metallothionein-2 is associated with metal accumulation and counteracts oxidative stress. Pedobiologia 54, 333–340.
Liebeke, M., Garcia-Perez, I., Anderson, C.J., Lawlor, A.J., Bennett, M.H., Morris, C.A., Kille, P., Svendsen, C., Spurgeon, D.J., Bundy, J.G., 2013. Earthworms produce phytochelatins in response to arsenic. PLOS One 8 (11), 81271–81283.
Lin, A.J., Zhang, X.Y., Chen, M.M., Cao, Q., 2007. Oxidative stress and DNA damages induced by cadmium accumulation. J. Environ. Sci. 19, 596–602.
Lin, D., Zhou, Q., Xie, X., Liu, Y., 2010. Potential biochemical and genetic toxicity of triclosan as an emerging pollutant on earthworms (*Eisenia fetida*). Chemosphere 81, 1328–1333.
Lin, D., Xie, X., Zhou, Q., Liu, Y., 2012a. Biochemical and genotoxic effect of triclosan on earthworms (*Eisenia fetida*) using contact and soil tests. Environ. Toxicol. 27, 385–392.
Lin, D., Zhou, Q., Xu, Y., Chen, C., Li, Y., 2012b. Physiological and molecular responses of the earthworm (*Eisenia fetida*) to soil chlortetracycline contamination. Environ. Pollut. 171, 46–51.
Liu, S., Zhou, Q., Wang, Y., 2011. Ecotoxicological responses of the earthworm *Eisenia fetida* exposed to soil contaminated with HHCB. Chemosphere 83, 1080–1086.
Liu, Y., Zhou, Q., Xie, X., Lin, D., Dong, L., 2010. Oxidative stress and DNA damage in the earthworm *Eisenia fetida* induced by toluene, ethylbenzene and xylene. Ecotoxicology 19, 1551–1559.
Lushchak, V.I., 2011. Environmentally induced oxidative stress in aquatic animals. Aquat. Toxicol. 101, 13–30.
Lydy, M.J., Linck, S.L., 2003. Assessing impact of triazine herbicides on organophosphate insecticide toxicity to the earthworm *Eisenia fetida*. Arch. Environ. Contam. Toxicol. 45, 343–349.
Maksymiec, W., Krupa, Z., 2006. The effects of short-term exposition to Cd, excess Cu ions and jasmonate on oxidative stress appearing in *Arabidopsis thaliana*. Environ. Exp. Bot. 57, 187–194.
Manier, N., Deram, A., Broos, K., Denayer, F.O., Wan Haluwyn, C., 2009. White clover modulation index in heavy metal contaminated soils—a potential bioindicator. J. Environ. Qual. 38, 685–692.
Manier, N., Brulle, F., Le Curieux, F., Vandenbulcke, F., Deram, A., 2012. Biomarker measurements in *Trifolium repens* and *Eisenia fetida* to assess the toxicity of soil contaminated with landfill leachate: a microcosm study. Ecotoxicol. Environ. Saf. 80, 339–348.
Mano, J., Ohno, C., Domae, Y., Asada, K., 2001. Chloroplastic ascorbate peroxidase is the primary target of methyl viologen-induced photooxidative stress in spinach leaves: its relevance to monodehydroascorbate radical detected with in vivo ESR. Biochim. Biophys. Acta 504, 275–287.
Marrs, K.A., 1996. Annu. Rev. Plant Physiol. Plant Mol. Biol. 47, 127–158.
Martinez, C.A., Loureiro, M.E., Oliva, M.A., Maestri, M., 2001. Differential responses of superoxide dismutase in freezing resistant *Solanum curtilobum* and freezing sensitive *Solanum tuberosum* subjected to oxidative and water stress. Plant Sci. 160, 505–515.
Martí, M.C., Camejo, D., Fernández-García, N., Rellán-Álvarez, R., Marques, S., Sevilla, F., Jiménez, A., 2009. Effect of oil refinery sludges on the growth and antioxidant system of alfalfa plants. J. Hazard. Mater. 171, 879–885.
Mascher, R., Lippmann, B., Holzinger, S., Bergmann, H., 2002. Arsenate toxicity: effects on oxidative stress response molecules and enzymes in red clover plants. Plant Sci. 163, 961–969.
Matés, J.M., Pérez-Gómez, C., De Castro, I.N., 1999. Antioxidant enzymes and human diseases. Clin. Biochem. 32, 595–603.
Mittler, R., Vanderauwera, S., Gollery, M., Van Breusegem, F., 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9, 490–498.
Mobin, M., Khan, N.A., 2007. Photosynthetic activity, pigment composition and antioxidative response of two mustard (*Brassica juncea*) cultivars differing in photosynthetic capacity subjected to cadmium stress. J. Plant Physiol. 164, 601–610.
Mohamed, A.A., Castagna, A., Ranieri, A., Sanità di Toppi, L., 2012. Cadmium tolerance in *Brassica juncea* roots and shoots is affected by antioxidant status and phytochelatin biosynthesis. Plant Physiol. Biochem. 57, 15–22.
Mohan, B.S., Hosetti, B.B., 1997. Potential phytotoxicity of lead and cadmium to *Lemna minor* grown in sewage stabilization ponds. Environ. Pollut. 98, 233–238.
Montalbini, P., 1992. Ureides and enzymes of ureide synthesis in wheat seeds and leaves and effect of allopurinol on *Puccinia recondita* f. sp. *tritici* infection. Plant Sci. 87, 225–231.
Moons, A., 2003. *Osgstu3* and *osgtu4*, encoding tau class glutathione S-transferases, are heavy metal- and hypoxic stress-induced and differentially salt stress-responsive in rice roots. FEBS Lett. 553, 427–432.
Moudouma, C., Gloaguen, V., Riou, C., Forestier, L., Saladin, G., 2012. High concentration of cadmium induces *AtPCS2* gene expression in *Arabidopsis thaliana* (L.) Heynh ecotype Wassilewskija seedlings. Acta Physiol. Plant. 34, 1083–1091.
Nakamori, T., Fujimori, A., Kinoshita, K., Ban-nai, T., Kubota, Y., Yoshida, S., 2010. mRNA expression of a cadmium-responsive gene is a sensitive biomarker of cadmium exposure in the soil collembolan *Folsomia candida*. Environ. Pollut. 158, 1689–1695.
Navascués, J., Pérez-Rontomé, C., Sánchez, D.H., Staudinger, C., Wienkoop, S., Rellán Álvarez, R., Becana, M., 2012. Oxidative stress is a consequence, not a cause, of aluminum toxicity in the forage legume *Lotus corniculatus*. New Phytol. 193, 625–636.
Navrot, N., Rouhier, N., Gelhaye, E., Jacquot, J.P., 2007. Reactive oxygen species generation and antioxidant systems in plant mitochondria. Physiol. Plant. 129, 185–195.
Neuhauser, E.F., Loehr, R.C., Milligan, D.L., Maleck, M.R., 1985. Toxicity of metals to the earthworm *Eisenia fetida*. Biol. Fert. Soils 1, 149–152.
Noriega, G., Balestrasse, K., Batlle, A., Tomaro, M., 2007. Cadmium induced oxidative stress in soybean plants also by the accumulation of δ-aminolevulinic acid. BioMetals 20, 841–851.
Organization for Economic Cooperation and Development (OECD), 1984a. OECD Guideline for Testing Chemicals. Section 2: Effects on Biotic Systems, Method, 207. Earthworm, Acute Toxicity Tests. Paris, France, 207 pp.
Organization for Economic Cooperation and Development (OECD), 1984b. Terrestrial Plants, Growth Test. Guidelines for Testing of Chemicals, no. 208. OECD, Paris.
Opdenakker, K., Remans, T., Keunen, E., Vangronsveld, J., Cuypers, A., 2012. Exposure of *Arabidopsis thaliana* to Cd or Cu excess leads to oxidative stress mediated alterations in MAPKinase transcript levels. Environ. Exp. Bot. 83, 53–61.
Owen, J., Hedley, B.A., Svendsen, C., Wren, J., Jonker, M.J., Hankard, P.K., Lister, L.J., Stürzenbaum, S.R., Morgan, A.J., Spurgeon, D.J., Blaxter, M.L., Kille, P., 2008. Transcriptome profiling of developmental and xenobiotic responses in a keystone soil animal, the oligochaete annelid Lumbricus rubellus. BMC Genomics 9, 266, http://dx.doi.org/10.1186/1471-2164-9-266.
Palmiter, R.D., 1998. The elusive function of metallothioneins. Proc. Natl. Acad. Sci. USA 95, 8428–8430.
Pan, S.-M., Chung, M.-H., 2002. The Character of Manganese Superoxide Dismutases in Arabidopsis. Submitted (SEP-2002) to the EMBL/GenBank/DDBJ databases.
Pan, Y., Wu, L.J., Yu, Z.L., 2006. Effect of salt and drought stress on antioxidant enzymes activities and SOD isoenzymes of liquorice (*Glycyrrhiza uralensis* Fisch). Plant Growth Regul. 49, 157–165.
Panda, S.K., Matsumoto, H., 2010. Changes in antioxidant gene expression and induction of oxidative stress in pea (*Pisum sativum* L.) under Al stress. BioMetals 23, 753–762.
Pandey, V., Dixit, V., Shyam, R., 2005. Antioxidative responses in relation to growth of mustard (*Brassica juncea* cv. *Pusa* Jaikisan) plants exposed to hexavalent chromium. Chemosphere 61, 40–47.
Paoletti, M.G., 1999. The role of earthworms for assessment of sustainability and as bioindicators. Agric. Ecosyst. Environ. 74, 137–155.
Parker, D.R., Norvell, W.A., 1999. Advances in so lution culture methods for plan t mine ral nutrie nt research. Adv. Agron. 65 (15), 1–313.
Paskova, V., Hilscherova, K., Feldmannova, M., Blaha, L., 2006. Toxic effects and oxidative stress in higher plants exposed to polycyclic aromatic hydrocarbons and their N-heterocyclic derivatives. Environ. Toxicol. Chem. 25 (12), 3238–3245.
Phang, I.C., Leung, D.W.M., Taylor, H.H., Burritt, D.J., 2011. The protective effect of sodium nitroprusside (SNP) treatment on *Arabidopsis thaliana* seedlings exposed to toxic level of Pb is not linked to avoidance of Pb uptake. Ecotoxicol. Environ. Saf. 74, 1310–1315.
Pinto, A.P., Alves, A.S., Candeias, A.J., Cardoso, A.I., de Varennes, A., Martins, L.L., Mourato, M.P., Gonçalves, M.L.S., Mota, A.M., 2009. Cadmium accumulation and antioxidative defences in *Brassica juncea* L. Czern, *Nicotiana tabacum* L. and *Solanum nigrum* L. Int. J. Environ. Anal. Chem. 89, 661–676.
Posmyk, M.M., Kontek, R., Janas, K.M., 2009. Antioxidant enzymes activity and phenolic compounds content in red cabbage seedlings exposed to copper stress. Ecotoxicol. Environ. Saf. 72, 596–602.
Puckett, E.E., Serapiglia, M.J., DeLeon, A.M., Long, S., Minocha, R., Smart, L.B., 2012. Differential expression of genes encoding phosphate transporters contributes to arsenic tolerance and accumulation in shrub willow (*Salix spp.*). Environ. Exp. Bot. 75, 248–257.
Puskas, F., Braun, L., Csala, M., Kardon, T., Marcolongo, P., Benedetti, A., Mandl, J., Banhegyi, G., 1998. Gulonolactone oxidase activity-dependent intravesicular glutathione oxidation in rat liver microsomes. FEBS Lett. 430, 293–296.
Raab, A., Feldmann, J., Meharg, A.A., 2004. The nature of arsenic-phytochelatin complexes in *Holcus lanatus* and *Pteris cretica*. Plant Physiol. 134, 1113–1122.
Radetski, C.M., Cotelle, S., Férard, J.F., 2000. Classical and biochemical endpoints in the evaluation of phytotoxic effects caused by the herbicide trichloroacetate. Environ. Exp. Bot. 44, 221–229.
Ramel, F., Sulmon, C., Cabello-Hurtado, F., Taconnat, L., Martin-Magniette, M.L., Renou, J.P., El Amrani, A., Couée, I., Gouesbet, G., 2007. Genome-wide interacting effects of sucrose and herbicide-mediated stress in *Arabidopsis thaliana*: novel insights into atrazine toxicity and sucrose-induced tolerance. BMC Genomics, 8.
Rea, P.A., Vatamaniuk, O.K., Rigden, D.J., 2004. Weeds, worms, and more. Papain's long-lost cousin, phytochelatin synthase. Plant Physiol. 136, 2463–2474.
Remans, T., Opdenakker, K., Guisez, Y., Carleer, R., Schat, H., Vangronsveld, J., Cuypers, A., 2012. Exposure of *Arabidopsis thaliana* to excess Zn reveals a Zn-specific oxidative stress signature. Environ. Exp. Bot. 84, 61–71.
Rhee, S.G., Chae, H.Z., Kim, K., 2005. Peroxiredoxins: a historical overview and speculative preview of novel mechanisms and emerging concepts in cell signaling. Free Radic. Biol. Med. 38, 1543–1552.

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Ribera, D., Narbonne, J.F., Arnaud, C., Saint-Denis, M., 2001. Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil, effects of carbaryl. Soil Biol. Biochem. 33, 1123–1130.

Rodriguez Milla, M.A., Maurer, A., Huete, A.R., Gustafson, J.P., 2003. Glutathione peroxidase genes in Arabidopsis are ubiquitous and regulated by abiotic stresses through diverse signaling pathways. Plant J. 36, 602–615.

Rodríguez-Serrano, M., Romero-Puertas, M.C., Zabalza, A.N.A., Corpas, F.J., Gómez, M., Del Río, L.A., Sandalio, L.M., 2006. Cadmium effect on oxidative metabolism of pea (*Pisum sativum* L.) roots. Imaging of reactive oxygen species and nitric oxide accumulation in vivo. Plant Cell Environ. 29, 1532–1544.

Russo, M., Sgherri, C., Izzo, R., Navari-Izzo, F., 2008. *Brassica napus* subjected to copper excess: phospholipases C and D and glutathione system in signalling. Environ. Exp. Bot. 62, 238–246.

Rombke, J., Jänsch, S., Didden, W., 2005. The use of earthworms in ecological soil classification and assessment concepts. Ecotoxicol. Environ. Saf. 62, 249–265.

Roxas, V.P., Smith, R.K., Allen, E.R., Allen, R.D., 1997. Nat. Biotechnol. 15, 988–991.

Rzepka, M.A., 2007. Mécanismes d'accumulation et de toxicité des composés organiques volatils chez les végétaux supérieurs. University of Lille p. 2 (Ph.D. degree report).

Saint-Denis, M., Narbonne, J.F., Arnaud, C., Thybaud, E., Ribera, D., 1999. Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil: effects of benzo(a)pyrene. Soil Biol. Biochem. 31, 1837–1846.

Saint-Denis, M., Narbonne, J.F., Arnaud, C., Ribera, D., 2001. Biochemical responses of the earthworm *Eisenia fetida andrei* exposed to contaminated artificial soil: effects of lead acetate. Soil Biol. Biochem. 33, 395–404.

Sanchez-Hernández, J.C., 2006. Earthworm biomarkers in ecological risk assessment. Rev. Environ. Contam. Toxicol. 188, 85–126.

Sanderson, M.A., Soder, K.J., Muller, L.D., Klement, K.D., Skinner, R.H., Goslee, S.C., 2005. Forage mixture productivity and botanical composition in pastures grazed by dairy cattle. Agron. J. 97, 1465–1471.

Sanderson, M.A., Brink, G., Ruth, L., Stout, R., 2012. Grass–legume mixtures suppress weeds during establishment better than monocultures. Agron. J. 104, 36–42.

Sawbridge, T., Ong, E.K., Binnion, C., Emmerling, M., Meath, K., Nunan, K., O'Neill, M., O'Toole, F., Simmonds, J., Wearne, K., Winkworth, A., Spangenberg, G., 2003. Generation and analysis of expressed sequence tags in white clover (*Trifolium repens* L.). Plant Sci. 165, 1077–1087.

Schrader, M., Fahimi, H.D., 2004. Mammalian peroxisomes and reactive oxygen species. Histochem. Cell Biol. 122, 383–393.

Schreck, E., Geret, F., Gontier, L., Treilhou, M., 2008. Neurotoxic effect and metabolic responses induced by a mixture of six pesticides on the earthworm *Aporrectodea caliginosa nocturna*. Chemosphere 71, 1832–1839.

Seeber, J., Seeber, G.U.H., Kossler, W., Langel, R., Scheu, S., Meyer, E., 2005. Abundance and trophic structure of macro-decomposers on alpine pastureland (Central Alps, Tyrol): effects of abandonment of pasturing. Pedobiologia 49, 221–228.

Semane, B., Cuypers, A., Smeets, K., Van Belleghem, F., Horemans, N., Schat, H., Vangronsveld, J., 2007. Cadmium responses in *Arabidopsis thaliana*: glutathione metabolism and antioxidative defence system. Physiol. Plant. 129, 519–528.

Seth, C.S., Kumar Chaturvedi, P., Misra, V., 2008. The role of phytochelatins and antioxidants in tolerance to Cd accumulation in *Brassica juncea* L. Ecotoxicol. Environ. Saf. 71, 76–85.

Shahandeh, H., Hossner, L.R., 2002. Role of soil properties in phytoaccumulation of uranium. Water Air Soil Pollut. 141, 165–180.

Shiyab, S., Chen, J., Han, F.X., Monts, D.L., Matta, F.B., Gu, M., Su, Y., Masad, M.A., 2009. Mercury-induced oxidative stress in Indian mustard (*Brassica juncea* L.). Environ. Toxicol. 24, 462–471.

Sies, H., 1991. Role of reactive oxygen species in biological processes. Klin Wochenschr 69, 965–968.

Singh, H.P., Kaur, G., Batish, D.R., Kohli, R.K., 2011. Lead (Pb)-inhibited radicle emergence in *Brassica campestris* involves alterations in starch-metabolizing enzymes. Biol. Trace Elem. Res. 144, 1295–1301.

Singh, J., Pillai, K.S., 1973. The world beneath us. Sci. Report. 1973, 318–321 (July).

Singh, S., Khan, N.A., Nazar, R., Anjum, N.A., 2008. Photosynthetic traits and activities of antioxidant enzymes in blackgram (*Vigna mungo* L. Hepper) under cadmium stress. Am. J. Plant Physiol. 3, 25–32.

Singh, S., Singh, S., Ramachandran, V., Eapen, S., 2010. Copper tolerance and response of antioxidative enzymes in axenically grown *Brassica juncea* (L.) plants. Ecotoxicol. Environ. Saf. 73, 1975–1981.

Sinha, S., Sinam, G., Mishra, R.K., Mallick, S., 2010. Metal accumulation, growth, antioxidants and oil yield of *Brassica juncea* L. exposed to different metals. Ecotoxicol. Environ. Saf. 73, 1352–1361.

Skipsey, M., Knight, K.M., Brazier-Hicks, M., Dixon, D.P., Steel, P.G., Edwards, R., 2011. Xenobiotic responsiveness of *Arabidopsis thaliana* to a chemical series derived from a herbicide safener. J. Biol. Chem. 286, 32268–32276.

Skórzyńska-Polit, E., Drążkiewicz, M., Krupa, Z., 2004. The activity of the antioxidative system in cadmium-treated *Arabidopsis thaliana*. Biol. Plant. 47, 71–78.

Smeets, K., Opdenakker, K., Remans, T., Van Sanden, S., Van Belleghem, F., Semane, B., Horemans, N., Guisez, Y., Vangronsveld, J., Cuypers, A., 2009. Oxidative stress-related responses at transcriptional and enzymatic levels after exposure to Cd or Cu in a multipollution context. J. Plant Physiol. 166, 1982–1992.

Smeets, K., Ruytinx, J., Semane, B., Van Belleghem, F., Remans, T., Van Sanden, S., Vangronsveld, J., Cuypers, A., 2008. Cadmium-induced transcriptional and enzymatic alterations related to oxidative stress. Environ. Exp. Bot. 63, 1–8.

Smirnoff, N., 2001. L-ascorbic acid biosynthesis. Vitam. Horm. 61, 241–266.

Smit, C.E., van Gestel, C.A.M., 1996. Comparison of the toxicity of zinc for the springtail Folsomia candida in artificially contaminated and polluted field soils. Appl. Soil Ecol. 3, 127–136.

Sobrino-Plata, J., Ortega-Villasante, C., Laura Flores-Cáceres, M., Escobar, C., Del Campo, F.F., Hernández, L.E., 2009. Differential alterations of antioxidant defenses as bioindicators of mercury and cadmium toxicity in alfalfa. Chemosphere 77, 946–954.

Song, Y., Zhu, L.S., Wang, J., Wang, J.H., Liu, W., Xie, H., 2009. DNA damage and effects on antioxidative enzymes in earthworm (*Eisenia foetida*) induced by atrazine. Soil Biol. Biochem. 41, 905–909.

Spurgeon, D.J., Hopkin, S.P., 1995. Extrapolation of the laboratory based OECD earthworm test to metal-contaminated field sites. Ecotoxicology 4, 190–205.

Spurgeon, D.J., Weeks, J.M., Van Gestel, C.A.M., 2003. A summary of eleven years progress in earthworm ecotoxicology. Pedobiologia 47, 588–606.

Spurgeon, D.J., Stürzenbaum, S.R., Svendsen, C., Hankard, P.K., Morgan, A.J., Weeks, J.M., Kille, P., 2004. Toxicological, cellular and gene expression responses in earthworms exposed to copper and cadmium. Comp. Biochem. Physiol. – C Toxicol. Pharmacol. 138, 11–21.

Stürzenbaum, S.R., Georgiev, O., Morgan, A.J., Kille, P., 2004. Cadmium detoxification in earthworms: from genes to cells. Environ. Sci. Technol. 38, 6283–6289.

Suzuki, N., Mittler, R., 2006. Reactive oxygen species and temperature stresses: a delicate balance between signaling and destruction. Physiol. Plant. 126, 45–51.

Szőllősi, R., Varga, I.S., Erdei, L., Mihalik, E., 2009. Cadmium-induced oxidative stress and antioxidative mechanisms in germinating Indian mustard (*Brassica juncea* L.) seeds. Ecotoxicol. Environ. Saf. 72, 1337–1342.

Taylor, S.A., Ashcroft, G.L., 1972. Physical edaphology: The physics of irrigated and non irrigated soils. San Francisco, W.H. Freeman.

Teisseire, H., Couderchet, M., Vernet, G., 1998. Toxic responses and catalase activity of *Lemna minor* L. exposed to folpet, copper, and their combination. Ecotoxicol. Environ. Saf. 40, 194–200.

Tsyusko, O.V., Hardas, S.S., Shoults-Wilson, W.A., Starnes, C.P., Joice, G., Butterfield, D.A., Unrine, J.M., 2012. Short-term molecular-level effects of silver nanoparticle exposure on the earthworm, *Eisenia fetida*. Environ. Pollut. 171, 249–255.

Unrine, J.M., Tsyusko, O.V., Hunyadi, S.E., Judy, J.D., Bertsch, P.M., 2010. Effects of particle size on chemical speciation and bioavailability of copper to earthworms (*Eisenia fetida*) exposed to copper nanoparticles. J. Environ. Qual. 39, 1942–1953.

Van Der Ploeg, M.J.C., Handy, R.D., Heckmann, L.H., Van Der Hout, A., Van Den Brink, N.W., 2013. C60 exposure induced tissue damage and gene expression alterations in the earthworm *Lumbricus rubellus*. Nanotoxicology 7 (4), 432–440.

Van Hook, R.I., 1974. Cadmium, lead and zinc distributions between earthworms and soils: potentials for biological accumulation. Bull. Environ. Contam. Toxicol. 12, 509–511.

van Wensem, J., Vegter, J.J., van Straalen, N.M., 1994. Soil quality criteria derived from critical body concentrations of metals in soil invertebrates. Appl. Soil Ecol. 1, 185–191.

Vanhoudt, N., Vandenhove, H., Opdenakker, K., Remans, T., Smeets, K., Martinez Bello, D., Van Hees, M., Wannijn, J., Vangronsveld, J., Cuypers, A., 2009. Induction of oxidative stress related responses in *Arabidopsis thaliana* following uranium exposure. Radioprotection 44, 191–196.

Vanhoudt, N., Vandenhove, H., Horemans, N., Wannijn, J., Bujanic, A., Vangronsveld, J., Cuypers, A., 2010a. Study of oxidative stress related responses induced in *Arabidopsis thaliana* following mixed exposure to uranium and cadmium. Plant Physiol. Biochem. 48, 879–886.

Vanhoudt, N., Vandenhove, H., Horemans, N., Wannijn, J., Van Hees, M., Vangronsveld, J., Cuypers, A., 2010b. The combined effect of uranium and gamma radiation on biological responses and oxidative stress induced in *Arabidopsis thaliana*. J. Environ. Radioact. 101, 923–930.

Vanhoudt, N., Vandenhove, H., Horemans, N., Remans, T., Opdenakker, K., Smeets, K., Bello, D.M., Wannijn, J., Van Hees, M., Vangronsveld, J., Cuypers, A., 2011a. Unraveling uranium induced oxidative stress related responses in *Arabidopsis thaliana* seedlings. Part I: responses in the roots. J. Environ. Radioact. 102, 630–637.

Vanhoudt, N., Cuypers, A., Horemans, N., Remans, T., Opdenakker, K., Smeets, K., Bello, D.M., Havaux, M., Wannijn, J., Van Hees, M., Vangronsveld, J., Vandenhove, H., 2011b. Unraveling uranium induced oxidative stress related responses in *Arabidopsis thaliana* seedlings. Part II: responses in the leaves and general conclusions. J. Environ. Radioact. 102, 638–645.

VDI, Verein Deutscher Ingenieure, 2000. Biologische Messverfahren zur Ermittlung und Beur-teilung der Wirkung von Luftverunreinigungen auf Pflanzen (Bioindikation). Verfahren der standardisierten Exposition von Grünkohl. – Biological Measuring Techniques for the Determination and Evaluation of Effects of Air Pollutants on Plants (Bioindication). Standardised Exposure of Green Cabbage. VDI-Guideline 3957 Part 3.

Verma, K., Shekhawat, G.S., Sharma, A., Mehta, S.K., Sharma, V., 2008. Cadmium induced oxidative stress and changes in soluble and ionically bound cell wall peroxidase activities in roots of seedling and 3-4 leaf stage plants of *Brassica juncea* (L.) czern. Plant Cell Rep. 27, 1261–1269.

Voroney, R.P., 2006. The soil habitat. In: Paul, Eldor A. (Ed.), Soil Microbiology, Ecology and Biochemistry. ISBN 0-12-546807-5.

Wang, C.Q., Zhang, Y.F., Zhang, Y.B., 2008. Scavenger enzyme activities in subcellular fractions of white clover (*Trifolium repens* L.) under PEG-induced water stress. J. Plant Growth Regul. 27, 387–393.

Wang, C.Q., Song, H., 2009. Calcium protects *Trifolium repens* L. seedlings against cadmium stress. Plant Cell Rep. 28, 1341–1349.

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Weisman, D., Alkio, M., Colón-Carmona, A., 2010. Transcriptional responses to polycyclic aromatic hydrocarbon-induced stress in *Arabidopsis thaliana* reveal the involvement of hormone and defense signaling pathways. BMC Plant Biol., 10.

Xie, X., Wu, Y., Zhu, M., Zhang, Y.K., Wang, X., 2011. Hydroxyl radical generation and oxidative stress in earthworms (*Eisenia fetida*) exposed to decabromodiphenyl ether (BDE-209). Ecotoxicology 20, 993–999.

Xiong, W., Bai, L., Muhammad, R.U.H., Zou, M., Sun, Y., 2012. Molecular cloning, characterization of copper/zinc superoxide dismutase and expression analysis of stress-responsive genes from *Eisenia fetida* against dietary zinc oxide. Comp. Biochem. Physiol. – C Toxicol. Pharmacol. 155, 416–422.

Xiong, W., Sun, Y., Zou, M., Muhammad, R.U.H., 2013. Molecular cloning, characterization of CAT, and eco-toxicological effects of dietary zinc oxide on antioxidant enzymes in *Eisenia fetida*. Environ. Sci. Pollut. Res. 20 (3), 1746.

Xue, Y., Gu, X., Wang, X., Sun, C., Xu, X., Sun, J., Zhang, B., 2009. The hydroxyl radical generation and oxidative stress for the earthworm *Eisenia fetida* exposed to tetrabromobisphenol A. Ecotoxicology 18, 693–699.

Yang, Y., Han, C., Liu, Q., Lin, B., Wang, J., 2008. Effect of drought and low light on growth and enzymatic antioxidant system of *Picea asperata* seedlings. Acta Physiol. Plant. 30, 433–440.

Yildiz, M., Terzi, H., Bingül, N., 2013. Protective role of hydrogen peroxide pretreatment on defense systems and BnMP1 gene expression in Cr(VI)-stressed canola seedlings. Ecotoxicology 22, 1303–1312.

Zaimoglu, Z., Koksal, N., Basci, N., Kesici, M., Gulen, H., Budak, F., 2011. Antioxidative enzyme activities in *Brassica juncea* L. and *Brassica oleracea* L. plants under chromium stress. J. Food Agric. Environ. 9, 676–679.

Zhang, Q., Zhu, L., Wang, J., Xie, H., Han, Y., Yang, J., 2012. Oxidative stress and lipid peroxidation in the earthworm *Eisenia fetida* induced by low doses of fomesafen. Environ. Sci. Pollut. Res., 1–8.

Zhang, W., Song, Y., Liu, M., Sun, T., 2009a. Effects of Benzo (a) pyrene on cytochrome P450 and antioxidant enzymes in earthworms. Liaoning Gongcheng Jishu Daxue Xuebao (Ziran Kexue Ban)/J. Liaoning Tech. Univ. (Natural Science Edition) 28, 846–848.

Zhang, X., Lu, Y., Shi, Y., Chen, C., Yang, Z., Li, Y., Feng, Y., 2009b. Antioxidant and metabolic responses induced by cadmium and pyrene in the earthworm *Eisenia fetida* in two different systems: contact and soil tests. Chem. Ecol. 25, 205–215.

Zheng, G., Lv, H.P., Gao, S., Wang, S.R., 2010. Effects of cadmium on growth and antioxidant responses in *Glycyrrhiza uralensis* seedlings. Plant Soil Environ. 56, 508–515.

Zheng, K., Liu, Z.T., Li, Y.J., Cui, Y.B., Li, M., 2013. Toxicological responses of earthworm (Eisenia fetida) exposed to metal-contaminated soils. Environ. Sci. Pollut. Res. Int. 20 (12), 8382–8390.

Zhou, G.K., Xu, Y., Li, J., Yang, L., Liu, J.Y., 2006. Molecular analyses of the metallothionein gene family in rice (Oryza sativa L.). J. Biochem. Mol. Biol. 39, 595–606.

Zhou, Z.S., Huang, S.Q., Guo, K., Mehta, S.K., Zhang, P.C., Yang, Z.M., 2007. Metabolic adaptations to mercury-induced oxidative stress in roots of *Medicago sativa* L. J. Inorg. Biochem. 101, 1–9.

Zhou, Z.S., Wang, S.J., Yang, Z.M., 2008. Biological detection and analysis of mercury toxicity to alfalfa (*Medicago sativa*) plants. Chemosphere 70, 1500–1509.

Zhou, Z.S., Guo, K., Elbaz, A.A., Yang, Z.M., 2009. Salicylic acid alleviates mercury toxicity by preventing oxidative stress in roots of *Medicago sativa*. Environ. Exp. Bot. 65, 27–34.

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