Bulletin of Environmental Contamination and Toxicology (2020) 105:255–260
https://doi.org/10.1007/s00128-020-02927-5

Biochemical Toxicity and Potential Detoxification Mechanisms in Earthworms *Eisenia fetida* Exposed to Sulfamethazine and Copper

Hong Rong[1,2] · Chengrun Wang[1,2] · Haitao Liu[1,2] · Min Zhang[1,2] · Yueting Yuan[1,2] · Yanjie Pu[1,2] · Jin Huang[1,2] · Jinyu Yu[1,2]

Received: 7 February 2020 / Accepted: 27 June 2020 / Published online: 6 July 2020

Abstract

The present study investigated the biochemical toxicity and potential detoxification mechanisms in earthworms *Eisenia fetida* exposed to sulfamethazine (SMZ) (7.5, 15 and 30 mg kg^{-1}) either alone or in combination with Copper (Cu) (100 mg kg^{-1}) in soil. The results showed that increasing concentrations of SMZ in soil activated superoxide dismutase, catalase and glutathione peroxidase isozymes, suggesting reactive oxygen species (ROS) burst in earthworms. Treatment with SMZ and Cu separately or in combination caused protein oxidation and damage, elevating the synthesis of ubiquitin, the 20S proteasome, cytochrome P450 (CYP450), and heat shock protein 70 (HSP70). Such treatments also induced the activities of proteases, endoproteinase (EP) and glutathione S-transferases (GSTs). The results suggested that the ubiquitin-20S proteasome, proteases, EP and HSP70 were involved in degradation or remediation of oxidatively damaged proteins. Elevated levels of CYP450 and GSTs also participated in the detoxification of the earthworms.

Keywords Earthworm (*Eisenia fetida*) · Antibiotic · Heavy metal · Oxidized protein · Ubiquitin-proteasome pathway

Sulfonamide antibiotics are widely applied for disease treatment in humans, animals or poultry, and are also a common feed additive. However, the utilization rate of such antibiotics is very low in humans and animals, and most enter the soil environment with manure in the form of the parent compound or its metabolites. In farmland soils of the Pearl River Delta and northeast of China, sulfonamide antibiotic levels have reached 321.4 μg kg^{-1} and 160.18 μg kg^{-1}, respectively, with a 100% detection rate (Li et al. 2011; An et al. 2015). Copper (Cu) is commonly added to animal feed as an additive along with sulfonamide antibiotics. Therefore, it often occurs in combination with sulfonamide antibiotics in the intestinal tract or excrement of livestock and poultry, and in turn can enter farmland soil after long-term fertilization with such manure.

✉ Chengrun Wang
chengrunwang@163.com

[1] School of Biological Engineering, Huainan Normal University, Huainan, China

[2] Key Laboratory of Bioresource and Environmental Biotechnology of Anhui Higher Education Institutes, Huainan Normal University, Huainan, China

It has been reported that the adsorption of antibiotics to minerals is due to binding of the antibiotics to divalent metal cations (Jia et al. 2008). The sorption mechanism to metals ions involves complexation reactions owing to the presence of many carboxyl, hydroxyl, amidogen, heterocyclic groups or electron donors in antibiotics (Zhao et al. 2018). Such complexation between antibiotics and metals may have synergistic effects on the inhibition of soil organisms (Kong et al. 2006; Zhang et al. 2012), and may change the toxicological effects of pollutants to different degrees. So far, there have been numerous studies on the ecological risks of single antibiotic or heavy metal pollutants on animals (Guo et al. 2017), plants (Xu et al. 2017) and microorganisms (Zhao et al. 2019). However, studies concerning the health risks for soil biota caused by the co-contamination of antibiotics and heavy metals are still limited.

Exposure to antibiotics or heavy metals has been shown to induce generation of reactive oxygen species (ROS) in animals (Guo et al. 2017; Kaushal et al. 2019). NADPH oxidase is the key enzyme involved in ROS generation, catalyzing the conversion of oxygen to superoxide radicals at the expense of NADPH (Sagi and Fluhr 2001). To mitigate oxidative damage caused by ROS, many antioxidant enzymes, such as catalase (CAT), ascorbate peroxidase (APX),

superoxide dismutase (SOD), guaiacol peroxidase (POD) and glutathione peroxidase (GPx), act together to scavenge excessive ROS (Mittler 2002). However, when ROS generation overwhelms the capacity of the antioxidant defense processes, damage may occur to intracellular proteins by oxidizing the amino acid side chains (e.g., forming carbonylated proteins). This in turn promotes protein accumulation or denaturation via covalent cross-linking, thereby leading to a loss of protein function or enzyme activity (Stadtman and Levine 2003; Grune et al. 2005).

The objective of the present study was to determine the biochemical toxicity and potential detoxification mechanisms in earthworms (*Eisenia fetida*) exposed to single treatment of sulfamethazine (SMZ), Cu or their combination in soil. The results may provide a scientific basis for assessment of the health risks of the combined effects of sulfonamide antibiotics and Cu on earthworms in soil and possible application of earthworms to remediate soils polluted by antibiotics and heavy metal(s).

Materials and Methods

Soil samples were taken from the campus of Huainan Normal University, Anhui Province, China. After air drying, the soil was ground and sieved through a 2 mm mesh, and 2 kg of the sifted soil was then placed in each container. The physicochemical properties and contents of representative heavy metals in the native soil were the same as previously reported by Wang et al. (2018). A stock solution of sulfamethazine (SMZ, Sigma) was prepared by dissolving a proper amount of SMZ into 0.1 M hydrochloric acid (HCl) solution. Moreover, Cu stock was prepared by directly dissolving a certain amount of $CuSO_4{\cdot}5H_2O$ (Sigma) into deionized water. The stock solutions were diluted and homogeneously sprayed into the soil respectively, followed by thoroughly blending. The concentrations of added SMZ and Cu were as follows: 0 (control, Ck), 7.5 mg kg^{-1} SMZ, 15 mg kg^{-1} SMZ, 30 mg kg^{-1} SMZ, 100 mg kg^{-1} Cu, 100 mg kg^{-1} Cu + 7.5 mg kg^{-1} SMZ, 100 mg kg^{-1} Cu + 15 mg kg^{-1} SMZ, and 100 mg kg^{-1} Cu + 30 mg kg^{-1} SMZ. Triplicates were conducted for each concentration, and 24 containers were prepared for each of two independent experiments.

After 1 week of equilibration at room temperature, contents of total Cu in the control and treated soils were measured according to the method as described by Wang et al. (2010). The contents of Cu were detected as 21.50 ± 2.35 mg kg^{-1} in the tested soils. SMZ residues in the tested soils were measured by the method of Lertpaitoonpan et al. (2015) with minor modifications. SMZ was not detected in the control and 100 mg/kg Cu-treated group. The contents of SMZ ranged from 0.382 to 2.776 mg kg^{-1} in the soils treated by SMZ alone, and from 0.221 to 3.432 mg kg^{-1} in the combined soils of SMZ and Cu (specific data not shown).

Moreover, sixteen earthworms (*Eisenia fetida*) with uniform size and clitellum were selected and transferred to each container according to OECD guidelines (2004). The earthworms were incubated at 21°C and 75% relative humidity for 30 days. The humidity of the soil was maintained at 30% (w/w) by adding an appropriate amount of deionized water after weighing each pot. Additionally, 0.05 kg of cow manure was spread over the soil surface in each pot. After 30 days' exposure, the earthworms were collected, thoroughly depurated and rinsed with deionized water, and then treated as described below.

Crude enzyme extraction was performed as described previously (Wang et al. 2018). The earthworms were homogenized on ice in an extraction buffer of 0.1 M Tris-HCl (pH 7.5) containing 0.25 M sucrose, 0.1 M EDTA, 0.2% (v/v) Triton X-100, 2 mM dithiothreitol, 1 mM phenylmethanesulfonyl fluoride, 5 mM ascorbic acid, 1 M benzamidine, 1 μg mL^{-1} leupeptin and 2 μg mL^{-1} aprotinin. Afterwards, the homogenates were centrifuged at 12,000 *g* for 10 min. The protein content of the supernatant was determined according to the method of Bradford (1976). All procedures were performed at 4°C and each treatment was performed in triplicate. SOD, APX and POD isozymes were detected based on methods described in García-Limones et al. (2002) and Wang et al. (2018). CAT isozyme was determined as described by Verma and Dubey (2003). An amount of crude enzyme extracted as described above was mixed with glycerin and bromophenol blue, and then loaded onto a polyacrylamide gel consisting of 4% stacking gel and 8% resolving gel. Native polyacrylamide gel electrophoresis was performed using a high-throughput Mini-PROTEAN 3 electrophoresis system (Bio-Rad) with voltages across the running stacking gel and resolving gel set at 85 and 140 V, respectively. GPx isozymes were detected according to the methods of Lin et al. (2002). A certain amount of crude protein was mixed with 2-mercaptoethanol, glycerol and bromophenol blue, and then separated on a polyacrylamide gel (4% stacking gel and 10% resolving gel). NADPH oxidase isozymes were assayed according to Sagi and Fluhr (2001). A certain amount of each sample was prepared in Tris-HCl (pH 7.8) buffer with 0.1% (w/v) 3-[(3-cholamidopropyl)-dimethyl-ammonio]-1-propane sulfonate (CHAPS) for 30 min at 45°C and then subjected to native polyacrylamide gel electrophoresis (4% stacking gel and 8% resolving gel). Endoproteinase (EP) isozymes were detected as described previously by Wang et al. (2018). Enzyme extracts were incubated at 37°C for 16 h and then loaded onto polyacrylamide gels (5% stacking gel and 8% resolving gel) containing 0.25% (w/v) gelatin.

Protease and glutathione S-transferases (GSTs) activities were determined as described by Gajewska and Skłodowska

(2010) and Habig and Jakoby (1981), respectively, with some modifications.

Total protein extraction and western blotting analysis were performed according to Wang et al. (2012, 2018). For western blotting analysis, the total protein extract was mixed with lysis buffer (0.5 M Tris pH 6.8 containing 20% (v/v) glycerol, 20% (w/v) SDS, 0.2% (w/v) bromophenol blue and 10% (v/v) β-mercaptoethanol), then heat-denatured by boiling for 5 min, cooled and centrifuged at 12,000 *g* for 2 min. Proteins were separated by SDS-PAGE (5% stacking gel and 12% resolving gel) and then transferred onto polyvinylidenefluoride (PVDF) membranes (Amersham). The transferred proteins were labeled with primary (1:3000) and secondary (1:10000) antibodies prior to visualization using a Super-Signal West Femto maximum sensitivity substrate (Thermo Scientific). The primary antibodies included anti-ubiquitin (Boster Corp.), anti-proteasome 20S β2 subunit (Enzo Life Sciences, Inc.), anti-HSP 70/HSC 70 (Sigma-Aldrich, USA), anti-CYP17A1 (Sangon Biotech Co., Ltd.) and anti-DNPH IgE (Sigma Aldrich, USA). Goat anti-rabbit or goat anti-mouse antibodies (Stressgen Corp.) were used as secondary antibodies. In addition, β-actin served as a sample loading control, labeled with primary antibody (anti-β-actin, diluted 1:2000, BM0626, Boster Corp.) and secondary antibody (goat anti-mouse, diluted 1:20,000, Stressgen Corp.). All loading samples were normalized to the β-actin level.

Statistically significant differences in the results were analyzed by one-way ANOVA followed by the *t*-test using the SPSS software package. Differences were considered significant at $p < 0.05$.

Results and Discussion

Previous studies have largely focused on single contamination by antibiotics or heavy metals and few studies have examined the underlying ecological toxicity in soil animals (e.g., earthworms) subjected to a combination of antibiotics and heavy metal(s) in soil. The present study investigated the biochemical toxicity and potential detoxification mechanisms in earthworms (*Eisenia fetida*) exposed to SMZ alone or in combination with Cu in soil.

The electrophoresis gel band intensities (denoting enzyme activities) indicated the total activities of isozymes. As shown in Fig. 1, under the single treatment of SMZ, the activities of SOD, CAT, GPx and NADPH oxidase increased with increasing SMZ in the soil. This suggested that increased concentrations of SMZ caused ROS overproduction in earthworms. When ROS accumulation exceeds the capacity of antioxidant defense systems, oxidatively damaged proteins are produced (e.g., carbonylated proteins) (Stadtman and Levine 2003; Grune et al. 2005). Immunoblotting showed the accumulation of

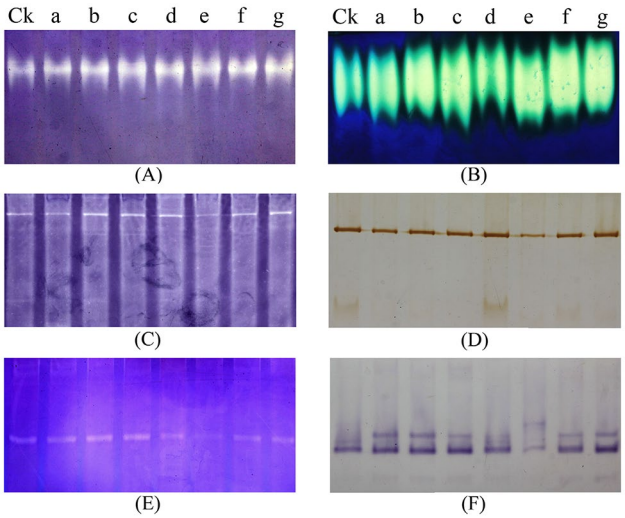

Fig. 1 Changes in isozyme patterns of SOD (**A**), CAT (**B**), APX (**C**), POD (**D**), GPx (**E**) and NADPH oxidase (**F**) enzymes in *Eisenia fetida* exposed to each treatment for 30 days. Ck denotes no treatment (control), whereas lowercase letters denote treatment with (a) 7.5 mg/kg SMZ; (b) 15 mg/kg SMZ; (c) 30 mg/kg SMZ; (d) 100 mg/kg Cu; (e) 100 mg/kg Cu + 7.5 mg/kg SMZ; (f) 100 mg/kg Cu + 15 mg/kg SMZ; (g) 100 mg/kg Cu + 30 mg/kg SMZ

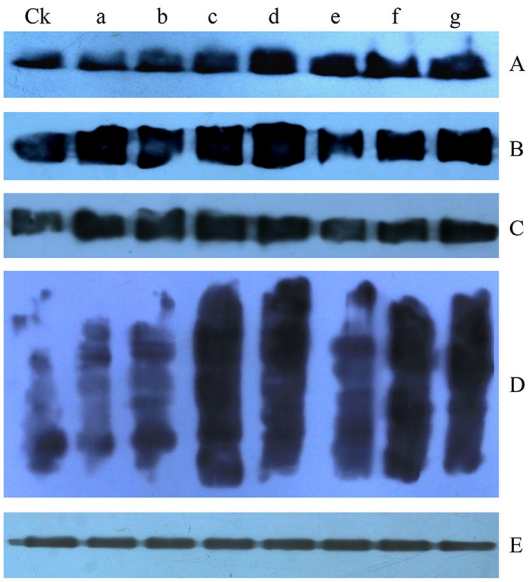

Fig. 2 Western blotting of ubiquitin (**A**), 20S proteasome (**B**), Hsp70 (**C**), carbonylated proteins (**D**) and β-actin (**E**) in *Eisenia fetida* exposed to each treatment for 30 days. Ck and a–g denote the same treatments mentioned in Fig. 1

carbonylated proteins at 7.5–30 mg kg^{-1} SMZ, indicating oxidative damage induced by SMZ in earthworms (Fig. 2d). Accumulation of these damaged proteins in cells can lead to cell death (Davies and Shringarpure 2006). Thus, elimination of oxidatively damaged proteins

is an important detoxification mechanism in earthworms exposed to SMZ in soil.

The present study showed that treatment with increasing concentrations of SMZ alone elevated the activities of SOD, CAT and GPx isozymes above those of controls (Fig. 1a, b and e), alleviating oxidative stress in the earthworms. Moreover, to eliminate abnormal proteins (e.g., oxidatively damaged proteins) in cells, several proteolytic systems have evolved in organisms. Among them, the ubiquitin-proteasome system is reported to be a major pathway for protein degradation (Smalle and Vierstra 2004). In this pathway, target proteins are first ubiquitinated by ubiquitin and then recognized and degraded by 26S proteasome (Glickman and Ciechanover 2002; Kahana 2007). The 26S proteasome consists of a catalytic core (20S proteasome) and two 19S regulatory subunits (Smalle and Vierstra 2004). As illustrated in Fig. 2a and b, levels of ubiquitin and the 20S proteasome exhibited similar trends: both tended to increase and were significantly enhanced at 15–30 mg kg^{-1} SMZ. Also, after treatment with Cu alone, both were distinctly induced compared with the controls. The synchronous change of ubiquitin and 20S proteasome indicated that the ubiquitin-20S proteasome pathway was mainly responsible for eliminating oxidatively damaged proteins in earthworms exposed to SMZ in soil.

In response to stress, organisms also increase the synthesis of heat shock proteins (HSP70) to repair or degrade denatured proteins (Roberts et al. 2010; Liu et al. 2019). In this study, HSP70 levels tended to increase with increasing SMZ and were markedly enhanced compared to the control under the single treatment of SMZ (Fig. 2c). These findings revealed that HSP70 was potentially involved in repairing or eliminating the oxidatively damaged proteins, thereby relieving the oxidative damage in earthworms.

Proteases also play an important role in the degradation of oxidized proteins (Knecht et al. 2009). Previous studies have reported that the proteolytic process is initiated by the 20S proteasome, which is then joined by proteases to eliminate oxidized proteins (Book et al. 2005; Polge et al. 2009). It was obvious from Fig. 3 that protease activities showed an increasing trend and were significantly elevated at 15–30 mg kg^{-1} under the single treatment of SMZ in soil ($p < 0.05$). Similarly, in comparison to the control, significant increases were observed in the integrated densities of EP isozyme bands (representing EP activities) under the treatment of SMZ alone (Fig. 3). This suggested that both pathways cooperated to eliminate oxidatively damaged proteins and were important detoxification mechanisms in earthworms.

Additionally, CYP450 and GSTs are involved in the metabolism or biotransformation of endogenous harmful substances in many organisms (Tompkins and Wallace 2007; Awali et al. 2019; Sun et al. 2019). With increasing concentrations of SMZ alone in soil, both GST activities and

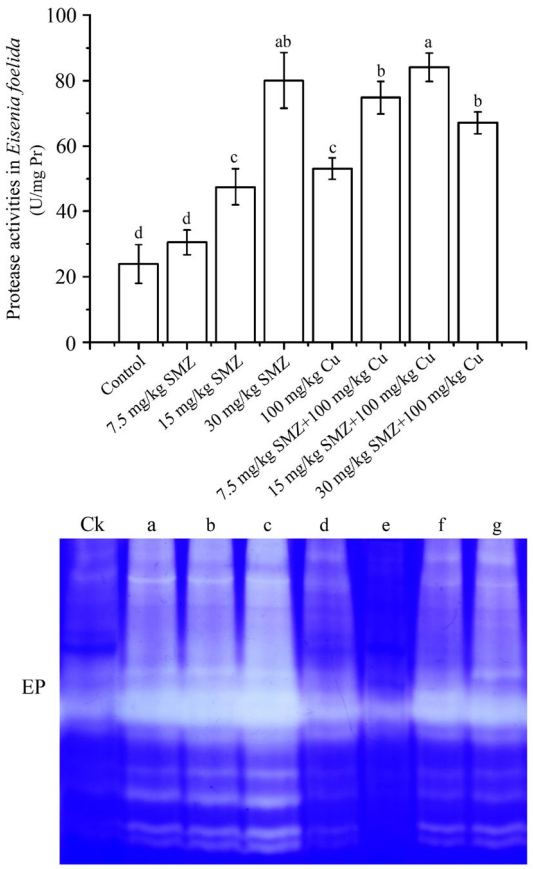

Fig. 3 Responses of protease and EP isozymes in *Eisenia fetida* exposed to each treatment for 30 days. Ck and a–g denote the same treatments mentioned in Fig. 1

CYP450 production were significantly increased above those of controls (Fig. 4), suggesting the involvement of CYP450 or GSTs in the detoxification mechanisms in earthworms.

When 7.5–30 mg kg^{-1} SMZ was added to Cu-polluted soil, the activities of SOD or CAT showed an upward tendency in comparison to Cu treatment alone, whereas APX, POD, GPx and NADPH oxidase decreased first and then rebounded in earthworms (Fig. 1). Meanwhile, the levels of ubiquitin and the 20S proteasome initially decreased and then increased with further increases of SMZ (Fig. 2a, b). Moreover, the activities of proteases and GSTs increased with increasing SMZ (Figs. 3 and 4). However, the products of ubiquitin, the 20S proteasome, HSP70 and CYP450 as well as activities of EP decreased for the combination with 7.5 mg kg^{-1} SMZ and then increased with higher SMZ concentrations of 15–30 mg kg^{-1} in the soil (Figs. 2, 3 and 4). From the above results, it could be concluded that enhanced activities of SOD, CAT, proteases and GSTs seemed to be important in alleviating oxidative stress and damage in earthworms exposed to lower doses of SMZ in combination with Cu.

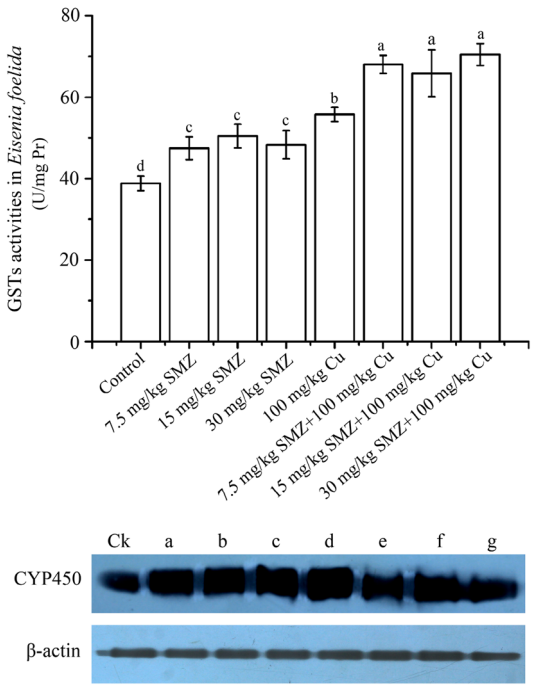

Fig. 4 Alterations in GST activities and CYP450 proteins in *Eisenia fetida* exposed to each treatment for 30 days. Ck and a–g denote the same treatments mentioned in Fig. 1

At higher SMZ concentrations in the combined treatment, these enzymes appeared to work together with the ubiquitin-20S proteasome, HSP70, CYP450 and EP isozymes to mitigate oxidative stress and eliminate oxidatively damaged proteins in earthworms.

In conclusion, increasing the concentration of SMZ in soils activated SOD, CAT and GPx isozymes, suggesting ROS burst in earthworms. Excessive ROS might be responsible for the accumulation of oxidatively damaged proteins (e.g., carbonylated proteins) in earthworms exposed to SMZ alone. The accumulation of carbonylated proteins elevated the production of CYP450 and HSP70, activities of proteases, EP and GSTs, and accelerated the ubiquitin-20S proteasome pathway, improving detoxification in earthworms. Under the co-exposure of SMZ and Cu, the activities of SOD, CAT, GSTs and proteases tended to increase, whereas the activities of POD, APX, GPx and EP, production of carbonylated proteins, CYP450, HSP70, ubiquitin, and the 20S proteasome first decreased then elevated with increasing SMZ. Thus, the activated SOD, CAT, GSTs and proteases alleviated oxidative stress and damage in earthworms exposed to low doses of SMZ in the combined treatments, and then these enzymes worked together with the ubiquitin-20S proteasome, HSP70, CYP450 and EP isozymes to mitigate oxidative stress and eliminate oxidatively damaged proteins in earthworms exposed to higher SMZ concentrations in the combination.

Therefore, the ubiquitin-20S proteasome, proteases, HSP70, GSTs, CYP450 and antioxidant enzymes (e.g., SOD, CAT and GPx) contribute to defense and detoxification systems in earthworms exposed to SMZ, Cu or their combination in soil.

Acknowledgements This work was supported by Foundation of the State Key Laboratory of Pollution Control and Resource Reuse of China (No. PCRRF19036), the fund from the Major Science and Technology Project of Anhui Province (No. 18030701189) and the Foundation of Scientific Research Project of Huainan Normal University, China (No. 2019XJZD02). We would also like to express our thanks to the anonymous reviewers for their constructive comments.

References

An J, Chen HW, Wei SH, Gu J (2015) Antibiotic contamination in animal manure, soil, and sewage sludge in Shenyang, northeast China. Environ Earth Sci 74:5077–5086

Awali S, Abdulelah SA, Crile KG, Yacoo KE, Almouseli A, Torres VC, Dayfeld DJ, Evans KR, Belanger RM (2019) Cytochrome P450 and glutathione-S-transferase activity are altered following environmentally relevant atrazine exposures in Crayfsh (*Faxonius virilis*). B Environ Contam Tox 103:579–584

Book AJ, Yang P, Scalf M, Smith LM, Vierstra RD (2005) Tripeptidyl peptidase II. An oligomeric protease complex from Arabidopsis. Plant Physiol 138:1046–1057

Bradford MM (1976) A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Davies KJ, Shringarpure R (2006) Preferential degradation of oxidized proteins by the 20S proteasome may be inhibited in aging and in inflammatory neuromuscular diseases. Neurology 66:S93–S96

Gajewska E, Skłodowska M (2010) Differential effect of equal copper, cadmium and nickel concentration on biochemical reactions in wheat seedlings. Ecotox Environ Safe 73:996–1003

García-Limones C, Hervás A, Navas-Cortés JA, Jiménez-Diaz RM, Tena M (2002) Induction of an antioxidant enzyme system and other oxidative stress markers associated with compatible and incompatible interactions between chickpea (*Cicer arietinum* L.) and *Fusarium oxysporum* f. sp. *Ciceris*. Physiol Mol Plant P 61:325–337

Glickman MH, Ciechanover A (2002) The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol Rev 82:373–428

Grune T, Merker K, Jung T, Sitte N, Davies KJ (2005) Protein oxidation and degradation during postmitotic senescence. Free Radical Bio Med 39:1208–1215

Guo H, Li KX, Wang W, Wang CG, Shen YC (2017) Effects of copper on hemocyte apoptosis, ROS production, and gene expression in White Shrimp *Litopenaeus vannamei*. Biol Trace Elem Res 179:318–326

Habig WH, Jakoby WB (1981) Assays for differentiation of glutathione S-transferase. Method Enzymol 77:398–405

Jia DA, Zhou DM, Wang YJ, Zhu HW, Chen JL (2008) Adsorption and cosorption of Cu(II) and tetracycline on two soils with different characteristics. Geoderma 146:224–230

Kahana C (2007) Ubiquitin dependent and independent protein degradation in the regulation of cellular polyamines. Amino Acids 33:225–230

Kaushal S, Ahsan AU, Sharma VL, Chopra M (2019) Epigallocatechin gallate attenuates arsenic induced genotoxicity via regulation of oxidative stress in balb/C mice. Mol Biol Rep 46:5355–5369

Knecht E, Aguado C, Cárcel J, Esteban I, Esteve JM, Ghislat G, Moruno JF, Vidal JM, Sáez R (2009) Intracellular protein degradation in mammalian cells: recent developments. Cell Mol Life Sci 66:2427–2443

Kong WD, Zhu YG, Fu BJ, Marschner P, He JZ (2006) The veterinary antibiotic oxytetracycline and Cu influence functional diversity of the soil microbial community. Environ Pollut 143:129–137

Lertpaitoonpan W, Moorman TB, Ong SK (2015) Effect of swine manure on sulfamethazine degradation in aerobic and anaerobic soils. Water Air Soil Pollut 226:81

Li YW, Wu XL, Mo CH, Tai YP, Huang XP, Xiang L (2011) Investigation of sulfonamide, tetracycline, and quinolone antibiotics in vegetable farmland soil in the Pearl River Delta area, southern China. J Agr Food Chem 59:7268–7276

Lin CL, Chen HJ, Hou WC (2002) Activity staining of glutathione peroxidase after electrophoresis on native and sodium dodecyl sulfate polyacrylamide gels. Electrophoresis 23:513–516

Liu BL, Fei F, Li XT, Wang XY, Huang B (2019) Effects of stocking density on stress response, innate immune parameters, and welfare of turbot (*Scophthalmus maximus*). Aquacult Int 27:1599–1612

Mittler R (2002) Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci 7:405–410

OECD (2004) Earthworm reproduction test (Eisenia fetidal Eisenia Andrei). Guideline for testing chemicals. No.222. OECD, Paris

Polge C, Jaquinod M, Holzer F, Bourguignon J, Walling L, Brouquisse R (2009) Evidence for the existence in *Arabidopsis thaliana* of the proteasome proteolytic pathway, activation in response to cadmium. J Biol Chem 284:35412–35424

Roberts RJ, Agius C, Saliba C, Bossier P, Sung YY (2010) Heat shock proteins (chaperones) in fish and shellfish and their potential role in relation to fish health: a review. J Fish Dis 33:789–801

Sagi M, Fluhr R (2001) Superoxide production by plant homologues of the gp91^{phox} NADPH oxidase. Modulation of activity by calcium and by tobacco mosaic virus infection. Plant Physiol 126:1281–1290

Smalle J, Vierstra RD (2004) The ubiquitin 26S proteasome proteolytic pathway. Annu Rev Plant Biol 55:555–590

Stadtman ER, Levine RL (2003) Free radical-mediated oxidation of free amino acids and amino acid residues in proteins. Amino Acids 25:207–218

Sun LL, Wang JN, Li XP, Cao CW (2019) Effects of phenol on glutathione S-transferase expression and enzyme activity in *Chironomus kiiensis* larvae. Ecotoxicology 28:754–762

Tompkins LM, Wallace AD (2007) Mechanisms of cytochrome P450 induction. J Biochem Mol Toxic 21:176–181

Verma S, Dubey RS (2003) Lead toxicity induces lipid peroxidation and alters the activities of antioxidant enzymes in growing rice plants. Plant Sci 164:645–655

Wang CR, Tian Y, Wang XR, Geng JJ, Jiang JL, Yu HX, Wang C (2010) Lead-contaminated soil induced oxidative stress, defense response and its indicative biomarkers in roots of *Vicia faba* seedlings. Ecotoxicology 19:1130–1139

Wang CR, Luo X, Tian Y, Xie Y, Wang SC, Li YY, Tian LM, Wang XR (2012) Biphasic effects of lanthanum on *Vicia faba* L. seedlings under cadmium stress, implicating finite antioxidation and potential ecological risk. Chemosphere 86:530–537

Wang CR, Rong H, Liu HT, Wang XF, Gao YX, Deng RH, Liu RY, Liu Y, Zhang D (2018) Detoxification mechanisms, defense responses, and toxicity threshold in the earthworm *Eisenia foetida* exposed to ciprofloxacin-polluted soils. Sci Total Environ 612:442–449

Xu YG, Yu WT, Ma Q, Zhou H, Jiang CM (2017) Toxicity of sulfadiazine and copper and their interaction to wheat (*Triticum aestivum* L.) seedlings. Ecotox Environ Safe 142:250–256

Zhang Y, Cai X, Lang X, Qiao X, Li X, Chen J (2012) Insights into aquatic toxicities of the antibiotics oxytetracycline and ciprofloxacin in the presence of metal: complexation versus mixture. Environ Pollut 166:48–56

Zhao FK, Yang L, Chen LD, Li SJ, Sun L (2018) Co-contamination of antibiotics and metals in peri-urban agricultural soils and source identification. Environ Sci Pollut Res 25:34063–34075

Zhao RX, Feng J, Liu J, Fu WJ, Li XY, Li B (2019) Deciphering of microbial community and antibiotic resistance genes in activated sludge reactors under high selective pressure of different antibiotics. Water Res 151:388–402