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Effect of manure compost on the herbicide prometryne bioavailability to wheat plants

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ABSTRACT

Soil amendment with manure compost may influence environmental behaviors and bioavailability of toxic organic chemicals (*e.g.* pesticide and polycyclic aromatic hydrocarbons). Dynamic parameters like adsorption, kinetics, mobility and degradation of pesticides have been intensively investigated. However, the current methods to evaluate the ultimate real bioavailability of pesticides to crops using physiochemical or biological approaches are limited. In this study, we developed a set of comprehensive and cost-effective parameters relevant to crop response to prometryne (s-triazine herbicide) to assess the accumulation and genotoxicity of the pesticide. Wheat plants exposed to 8 mg kg^{-1} prometryne for 10 d showed stunt growth, reduced chlorophyll content and damaged membrane lipid. Concomitant treatment with 5% pig manure compost (PMC) alleviated the toxic effect on the plant. Prometryne in soils was readily accumulated by wheat. However, such an accumulation was significantly inhibited by PMC application. Because excessively accumulated prometryne triggered oxidative damage to plants, the biochemical responses of several antioxidant enzymes along with their molecular expressions were determined. In most cases, the activities and transcriptional expression of the enzymes were activated upon the exposure to prometryne but the process was prevented by PMC application. The set of biological parameters tested in this study were very sensitive and cost-effective, and therefore can be used to evaluate the degree of pesticide contamination to plants and other organisms.

1. Introduction

Modern agriculture requires substantial input of pesticides to soils to control weeds and insects. While the pesticide usage in farmland has brought great benefits to crop production, it simultaneously generates great concerns linked to the contamination to ecosystem and food safety. In fact, contamination of pesticide (or their residue) to soils has become one of the environmental problems throughout the world [1]. A number of pesticides and its metabolites have been recently detected as contaminants in soil–plant–water systems [2–4]. Prometryne [2,4-bis (isopropylamino)-6-(methylthio)-s-triazine] is a selective herbicide of the s-triazine chemical family and substantially utilized as a controller of annual grasses in China and other developing countries. On the basis of the classification [5], its K_{oc} value varies between 311 and 614, indicating that prometryne is moderately mobile in soils. As prometryne is usually soil-applied and relatively water soluble, it tends to accumulate in crops [6]. Recent studies have demonstrated that prometryne was released into surface or even ground water [7], but environmental assessment of the pesticide behavior and bioavailability is restricted to physiochemical identification. Environmentally relevant evaluation needs a comprehensive analysis of biological and physicochemical impacts of the pesticide on soil ecosystem.

The usage of compost derived from domestic sewage sludge, wastewater residuals, animal manure or plant debris is one of the traditional agronomic practices in China owning to its beneficial nutrient recycling and improvement of soil quality [8–10]. Such an economically sound management has been long appreciated and represents a major effort to maintain the quality of soils. Enrichment of soil organic matter indeed sustains water holding capacity, making plants less prone to the condition of dry weather [8]. However, successive addition of compost to soils may generate adverse effects such as accumulation of components toxic to crops or soils (*e.g.* ammonia from animal manures, soluble salts and pathogens microorganisms), restriction of the seed germination and inhibition of plant growth [11]. More importantly, the environmental behavior of pesticides in soils is likely to be altered by organic matter because increased soil organic compounds may affect many aspects of physicochemical properties of pesticides [12]. Specifically,

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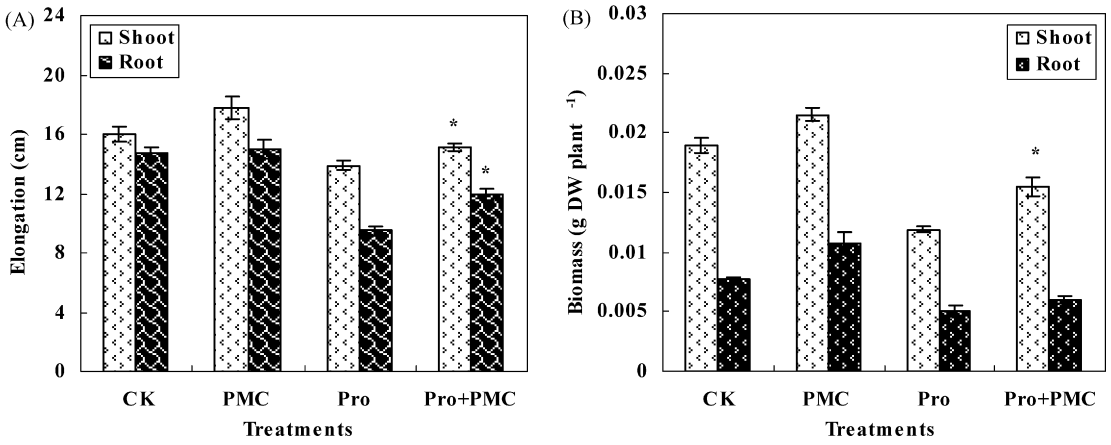

Fig. 1. Effect of prometryne on the growth of wheat treated with or without pig manure compost (PMC). CK: control (PMC-free); PMC: soil + 5% PMC; Pro: soil + prometryne (8 mg kg^{-1}); and Pro + PMC: 5% PMC + prometryne (8 mg kg^{-1}). Values are the means ± SD (n = 3). Asterisks indicate that mean values are significantly different between the treatments of Pro + PMC and Pro (P < 0.05).

dissolved organic matter (DOM) derived from solid organic matter or others reduced pesticide sorption through stable DOM–pesticide interactions or by competing with pesticide molecules for the sorption sites on the soil [13–15]. In contrast, the pesticide sorption may be also enhanced if the DOM sorbed on soils provides additional sites for pesticide sorption [16]. The paradoxical results are frequently reported and the overall effect of both soluble and insoluble DOM on pesticide sorption/desorption is not easy to predict [12]. Sufficient research is needed to investigate the optimal timing and rates of compost application. In recent years, great efforts have been made to assess the consequences of exogenous sources of organic carbon on the behavior of pesticides in soils [11,17]. Thus far, a majority of studies have dealt with the effect of composts on the environmental fate of pesticides/herbicides. Relatively few were reported on the effect of composts on bioavailability and ecotoxicology of soil pesticides. To ensure that successive application of compost is properly managed, there is a need to estimate the effect of compost on bioavailability of pesticides in soil and plant environment. Therefore, the objective of this study was to (i) evaluate the effect of compost applications on bioavailability of soil prometryne to crops and bioaccumulation of prometryne in crops and (ii) seek a set of sensitive biomarkers for diagnosing potential effects of compost on ecotoxicology of the herbicide. The study will assist the establishment of strategies of compost application and the reduction of the environmental risks of the herbicide to crop production and potential phytoremediation [2].

2. Materials and methods

2.1. Materials and treatments

The structure and some properties of prometryne have been described previously [7]. The pig manure compost (PMC) collected from a local farm near Nanjing was sieved through a 2.0 mm mesh, autoclave-treated and stored at 4 °C prior to use. Growth and treatments of wheat plants were described previously [6].

Intact soils (Eutric gleysols, N 32.03°; E 118.84°) were collected from the surface layer (0–20 cm). The soil was subject to autoclave treatment prior to use. Aliquots of soils were mixed with prometryne and/or PMC. Prometryne was added to the soil at 8 mg kg^{-1} (the dosage used in practice). The autoclave-treated pig manure compost was used at the dose of 5%, corresponding to the agronomic dose of 30 t ha^{-1} (based on the field application to a depth of 30 cm). The samples were blended thoroughly in a rotary shaker for 24 h. The composition and physiochemical properties of PMC alone and PMC (5%) + soil were summarized in Supplemental Tables S1 and S2.

2.2. Determination of physiological parameters

Chlorophyll and cellular lipid peroxides were quantified using the tissue extraction and spectrophotometrical assays [6,18]. The content of superoxide anion free radical ($O_2^{\bullet -}$) were visualized in wheat leaves using nitroblue tetrazolium (NBT) as a substrate according to methods by Zhou et al. [18].

2.3. Enzyme activity assay

Activities of superoxide dismutase (SOD, EC 1.15.1.1) were assayed by measuring its capacity of inhibiting the photochemical reduction of nitroblue tetrazolium (NBT) [19]. Catalase (CAT, EC 1.11.1.6) activity was assayed by consumption of H_2O_2 in 3 mL volume reaction medium of 50 mM of potassium phosphate buffer, 1% H_2O_2 and 50 μL enzyme extract [20]. Guaiacol peroxidase (POD, EC 1.11.1.7) activity was assayed on the base of guaiacol oxidation using hydrogen peroxide [21]. Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured by observing the oxidative rate of H_2O_2-dependent ascorbate [22]. Glutathione S-transferase (GST, EC 2.5.1.18) activity was assayed according to the method of Yin et al. [23]. Glutathione reductase (GR, EC 1.6.4.2) were assayed spectrophotometrically by tracing the decrease in absorbance at 340 nm and 25 °C due to the oxidation of NADPH [18].

2.4. Transcript analysis and DNA degradation

Total RNA was extracted from 100 mg of fresh tissues in liquid nitrogen using Trizol reagent (Invitrogen, Carls-

Table 1
Effect of PMC on prometryne accumulation in soils and wheat. Pro: 8 mg kg^{-1} prometryne only; Pro + PMC: 8 mg kg^{-1} prometryne + 5% pig manure compost.

Treatments	Soil (mg kg^{-1} dry soil)	Shoot (mg kg^{-1} Fresh plant)	Root (mg kg^{-1} Fresh plant)	Shoot BCF	Root BCF	TF
Pro	2.744 ± 0.013b	6.681 ± 0.024a	2.747 ± 0.017a	2.434 ± 0.003a	1.001 ± 0.002a	2.432 ± 0.002a
Pro + PMC	2.881 ± 0.012a	3.533 ± 0.016b	1.833 ± 0.007b	1.226 ± 0.002b	0.636 ± 0.001b	1.927 ± 0.002b

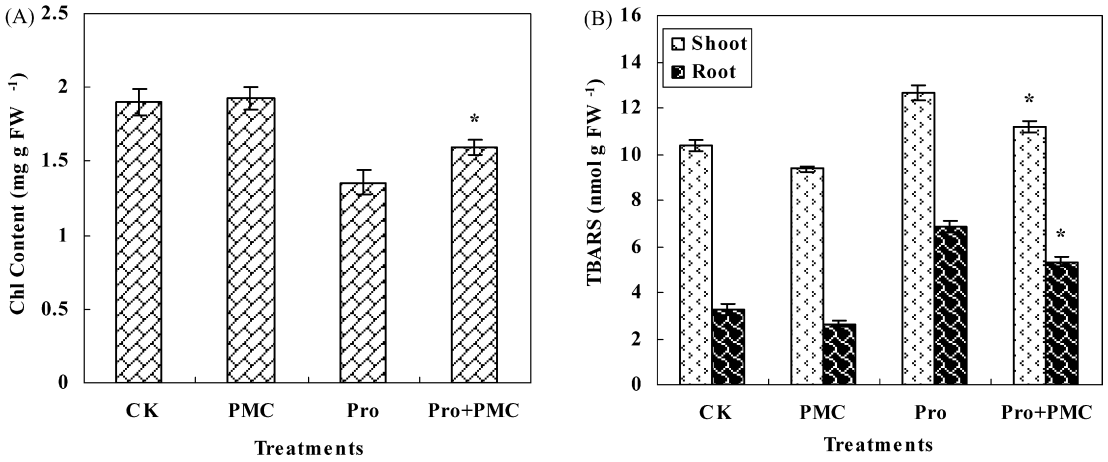

Fig. 2. Effects of prometryne on contents of chlorophyll (A) and TBARS (B) in wheat treated with or without PMC. CK: control (PMC-free); PMC: soil + 5% PMC; Pro: soil + prometryne (8 mg kg^{-1}); and Pro + PMC: 5% PMC + prometryne (8 mg kg^{-1}). Values are the means ± SD (n = 3). Asterisks indicate that mean values are significantly different between the treatments of Pro + PMC and Pro (P < 0.05).

bad, CA). Reverse transcription was carried out at 42 °C in the 25 μL reaction mixture containing 3 μg RNA, 0.5 μg oligo (dT) primers [5′-GTGCCCATCGGCGTGCTTCT-3′ (sense); 5′-CGTGTTGCGCTTGATGTGGC-3′ (antisense)], 12.5 nmol dNTPs, 12.5 units of RNase inhibitor and 5 units of AMV reverse transcriptase (Takara). The primer sequences of *Cu/Zn-SOD* and *GST* were designed based on the GenBank Database (NCBI) [6]. The first strand cDNA served as a template for polymerase chain amplification and was used to analyze transcripts. RT-PCR products were obtained after 30 PCR cycles. DNA isolation and laddering were assayed using cetyltrimethylammonium bromide (CTAB) as described previously [24].

2.5. Prometryne extraction and analysis

Prometryne in the soil was analyzed with following steps: (a) the sample was ultrasonicated for 30 min in the presence of acetone; (b) the extract solution was concentrated into 3 mL acetone; (c) the samples was transferred onto a LC-C_{18} column, followed by 5 mL of mixture (petroleum ether/ether, 98:2, V/V) passed through the column at 2.5 mL min^{-1}. The remaining section in the column was eluted with 30 mL acetone at a flow rate of 1 mL min^{-1}; and (d) 1 mL prometryne sample in methanol was quantified by HPLC. The similar procedures were performed for prometryne analysis in plant tissues, except for the first step of extraction with 40 mL mixed acetone–water (3:1, V/V).

2.6. Statistical analysis

Each result shown in the paper was the mean of at least three replicated treatments. Significance of differences between the treatments was statistically evaluated by standard deviation and Student's t-test methods.

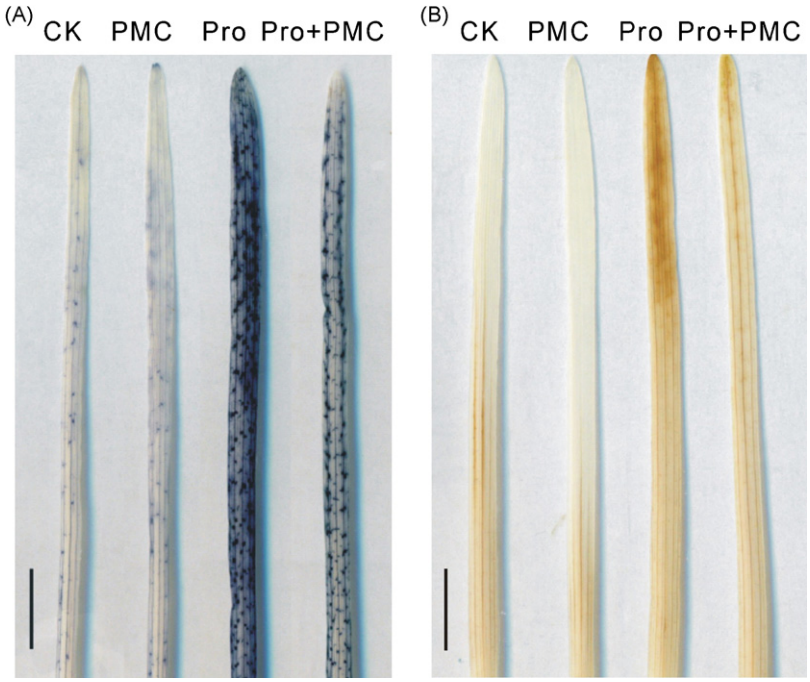

Fig. 3. Effects of prometryne on superoxide radical accumulation (A) and hydrogen peroxidase accumulation (B) in wheat plants with or without PMC. CK: control (PMC-free); PMC: soil + 5% PMC; Pro: soil + prometryne (8 mg kg^{-1}); and Pro + PMC: 5% PMC + prometryne (8 mg kg^{-1}). The scale bar in the graph indicates 10 mm.

3. Results and discussion

3.1. Growth response to prometryne in the presence of PMC

To investigate the impact of PMC on wheat growth under prometryne exposure, the growth responses to prometryne and the manure compost were first investigated. Treatment with 8 mg kg^{-1} prometryne for 10 d had an inhibitory effect on the root and shoot growth (Fig. 1). The inhibition of root elongation with prometryne was more pronounced that that of shoot (Fig. 1A). Both root and shoot growth was severely inhibited by prometryne exposure (Fig. 1B). However, simultaneous treatment with 5% pig manure compost (PMC) for 10 d increased the elongation and biomass of the plants. These results indicated that PMC improved plant growth under prometryne exposure. Organic amendments can induce an increase in plant-available nutrients and soil organic matter [8].

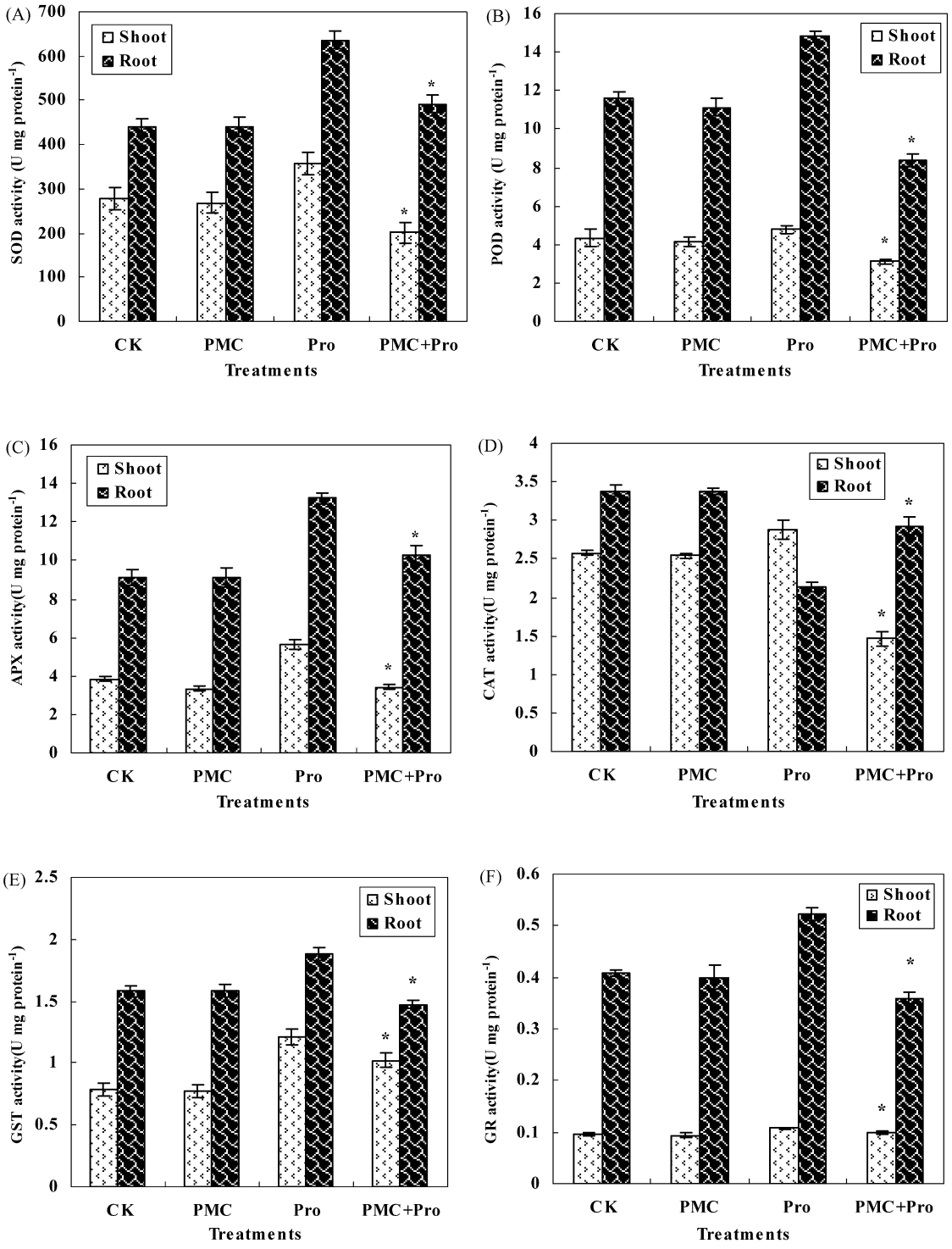

Fig. 4. Effects of prometryne on the activities of SOD (A), POD (B), APX (C), CAT (D), GST (E) and GR (F) in wheat plants treated with or without PMC. CK: control (PMC-free); PMC: soil + 5% PMC; Pro: soil + prometryne (8 mg kg^{-1}); and Pro + PMC: 5% PMC + prometryne (8 mg kg^{-1}). Values are the means ± SD ($n = 3$). Asterisks indicate that mean values are significantly different between the treatments of Pro + PMC and Pro ($P < 0.05$).

Under the condition, the plant roots may benefit from the uptake of nutrients released from the compost. The present study indicated that the beneficial effect from PMC may result from the improvement of growth of plants exposed to prometryne.

3.2. Bioaccumulation of prometryne in the presence of PMC

To get insights into the process of PMC effect, we determined accumulation of prometryne in wheat plants. Prometryne in untreated soil was undetectable (data not shown). Addition of prometryne to the soil at 8 mg kg^{-1} resulted in substantial accumulation of prometryne in wheat (Table 1). The shoot accumulated much more prometryne than the root. Interestingly, such accumulation could be greatly reduced by addition of PMC. The prometryne concentrations in shoots and roots decreased to 52.9% and 66.7% of the controls (prometryne treatment alone), respectively. These results indicated that prometryne bioaccumulation in wheat crops

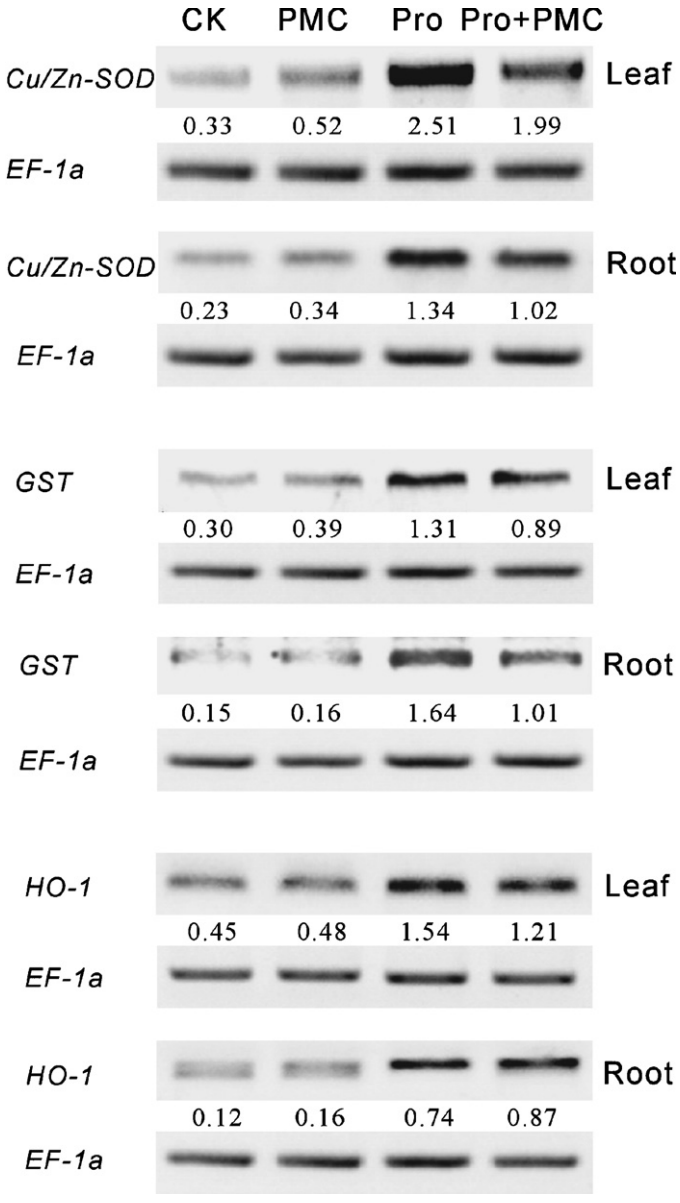

Fig. 5. Effects of prometryne and pig manure compost (PMC) on transcript abundance of *Cu/Zn-SOD* (A), *GST* (B) and *HO-1* (C) in wheat. CK: control (PMC-free); PMC: soil + 5% PMC; Pro: soil + prometryne (8 mg kg^{-1}); and Pro + PMC: 5% PMC + prometryne (8 mg kg^{-1}). *EF-1a* was used for cDNA normalization.

was significantly reduced in the soil amended with pig manure compost. Notably, application of PMC did not result in the degradation of prometryne in the soil. In contrast, some organic pollutants were accumulated in plants in the presence of small molecule weight organic compounds [25].

Bioconcentration factor (BCF) is defined as the quotient between the organism and medium substance concentrations [26]. The BCF values for prometryne treatment alone were significantly lower than those with Pro + PMC, suggesting that addition of PMC to the soil reduced the capacity of prometryne translocation from the soil to tissues. Translocation factor (TF) reflects the ratio of prometryne concentration in shoots to roots and can be used to evaluate the plant capability for accumulating prometryne. The TF value for Pro was significantly higher than that of Pro + PMC, indicating that addition of PMC reduced the translocation of prometryne from roots to shoots.

Addition of compost to soils increases the amount of DOM that may influence the binding, transport and bioavailability of pesticides [27,28]. With regard to the pesticide binding, early study showed that organic amendments promoted adsorption of pesticides and thereby reduced the bioavailability [29]. In contrast, recently studies have demonstrated that organic amendments reduced organic pollutant sorption onto soils and promoted desorption of pesticides from soils [28,30], and thereby increased the bioavailability of some hydrophobic organic contaminants [15]. The paradoxical results may result from the varied experimental conditions including the inherent properties of organic amendments and pesticides, soil properties, timing and pattern of organic matter usage, and many other environmental factors.

Amongst the factors, soil amendments are most likely to play a centre role in modulating the mobility and decay of the soil-applied pesticides. To date, a number of estimations of pesticide bioavailability has been documented in both batch and column studies [6,15,26,27]. However, examination of the apparent bioavailability of pesticide is insufficiently representative of the real bioavailability to plants. Only assessment of its bioaccumulation in plants would represent the real interaction between the organic amendment and pesticide. The present study determined the accumulation of prometryne in the major crop wheat in the presence of PMC. The possible reason for the reduced accumulation of prometryne with PMC may result from following possibilities: (a) with the addition of PMC to soils, the retention of the pesticide on soil particles increased and consequently reduced availability of prometryne to plants and (b) PMC would interact with prometryne in the form that limited its uptake by plants. However, the precise mechanism underlining the effect of PMC on organic contaminants in soils remains to be elucidated.

3.3. Effect of PMC on metabolite accumulation in the presence of prometryne

Our previous studies have demonstrated that the reduction of chlorophyll content is a sensitive biomarker to monitor the damage of pesticides to plants [23]. As shown in Fig. 2A, addition of PMC significantly improved the content of chlorophyll, compared to the treatment with prometryne alone. This result indicated that PMC improved photosynthesis and growth of leaves.

Over-generation of reactive oxygen species (ROS) is a rapid and sensitive response of plants to environmental stimuli [6,31,32]. Amongst ROS, $O_2^{\bullet -}$ and H_2O_2 were used to illustrate the degree of oxidative injury to cells. Histochemical staining of O_2^- with nitroblue tetrazolium (NBT) showed that treatment with prometryne-induced intense staining in tissues (Fig. 3A), indicating a higher level of O_2^- produced in leaves. However, simultaneous treatment with PMC-suppressed the O_2^- production. Hydrogen peroxidase (H_2O_2) is another species of major ROS which also

damage many biomolecules when over-produced in cells [18,23]. H_2O_2 can be visually detected in leaves using 3,3-diaminobenzidine (DAB). The leaves treated with prometryne alone were stained extensively, and those pretreated with PMC had light staining (Fig. 3B), indicating that organic amendments exerted a protective effect on the plant against prometryne toxicity. Because high abundance of ROS cells is directly linked to the oxidative damage to biomolecules [23,31], we therefore determined the membrane lipid peroxides, expressed as thiobarbituric acid reactive substances (TBARS). Fig. 2B illustrates the higher level of TBARS in prometryne-exposed seedlings than that of the untreated seedlings. Again, PMC amendment reduced significantly the production of TBARS. Compared to the control, the average values of TBARS with PMC decreased by 11.7% in shoots and 22.3% in roots, respectively.

3.4. Using a group of enzymes as biomarkers responding to prometryne with PMC

Plants have developed a well-organized antioxidative defense system comprising enzymatic antioxidants to scavenge ROS. Activity of antioxidant enzymes, reflecting the ROS pool, is often used as a biomarker for various abiotic stresses [32–36]. Regulation of ROS abundance can be achieved by a group of enzymes such as SOD, CAT and APX [7,21]. The total activities of SOD were drastically increased upon the exposure of plants to 8 mg kg^{-1} prometryne (Fig. 4A). However, SOD activities in prometryne-treated roots were significantly decreased by PMC, suggesting that PMC depressed the activity of SOD under the prometryne exposure.

Of the diverse antioxidative enzymes involved in the elimination of ROS, POD is another indicator of oxidative damage to plants; it participates in the breakdown of H_2O_2 and lignin biosynthesis in the presence of H_2O_2 [36]. APX is one type of POD but uses ascorbate as electron donor in the first step in the ascorbate–glutathione cycle to remove H_2O_2 [20]. In this study, both POD and APX displayed a pattern of activities similar to SOD (Fig. 4B and C). CAT is one of the key enzymes involved in the removal of toxic hydrogen peroxides. Its activity was depressed in roots with prometryne but was greatly recovered by addition of PMC (Fig. 4D). CAT in shoots showed a pattern of activity opposite to that in roots. The activity GST can be induced by a variety of stimuli [37]. GST catalyses the conjugation of glutathione to several electrophilic substrates and the complex formed is sequestrated. GST activities in wheat were enhanced by prometryne exposure. Treatment with PMC in the presence of prometryne reduced the GST activity (Fig. 4E). Increased GST activities were reported under the herbicide stress in wheat [23,37]. However, no report has been available indicating the regulatory process of herbicide-induced toxicity by organic amendments.

GR is localized mainly in the chloroplast in which it represents about 80% of total GR activities in leaf tissues; it also can be found in cytosol, glyoxysomes and peroxisomes [38]. Like APX, GR is one of the major components in the ascorbate–glutathione cycle, by which the efficient recycling of glutathione is ensured by GR. Hence, GR plays a critical role in protecting plants against oxidative stress. The present study showed that the GR activity in roots was greatly enhanced by the prometryne exposure but simultaneous treatment with PMC reduced the activity to the basal level (Fig. 4F). No difference of the GR activities between the Pro and PMC + Pro treatments was observed in shoots. It has been observed that plants under environmental stimuli tend to have high activities of GR [33]. PMC-suppressed GR activity suggests the amelioration of prometryne toxicity to wheat plants.

3.5. Gene expression in response to prometryne in the presence of PMC

Previous studies indicate that a variety of genes can be induced by xenobiotics [39–41]. To confirm the response of enzymes to prometryne, a RT-PCR-based assay was carried out to determine the transcript abundance of *Cu/Zn-SOD* and *GST*. We treated the transcriptional parameters as potential molecular markers to estimate genotoxicity generated by the pesticide. Expressions of *Cu/Zn-SOD* and *GST* were up-regulated by prometryne exposure (Fig. 5). The molecular responses of *Cu/Zn-SOD* and *GST* genes in leaves with 8 mg kg^{-1} prometryne increased 7.61 and 4.37-folds, respectively, as compared to the control (CK). In similar, expressions of the two genes with prometryne were blocked by PMC administration. The molecular method appeared sensitive and efficient to assess the existing of pesticides in plants.

We further determined the transcripts of *HO-1* (coding heme oxygenase-1, EC 1.14.99.3) because HO-1 provides an excellent system in organisms for understanding inducible gene expression upon oxidative stress triggered by many other environmental

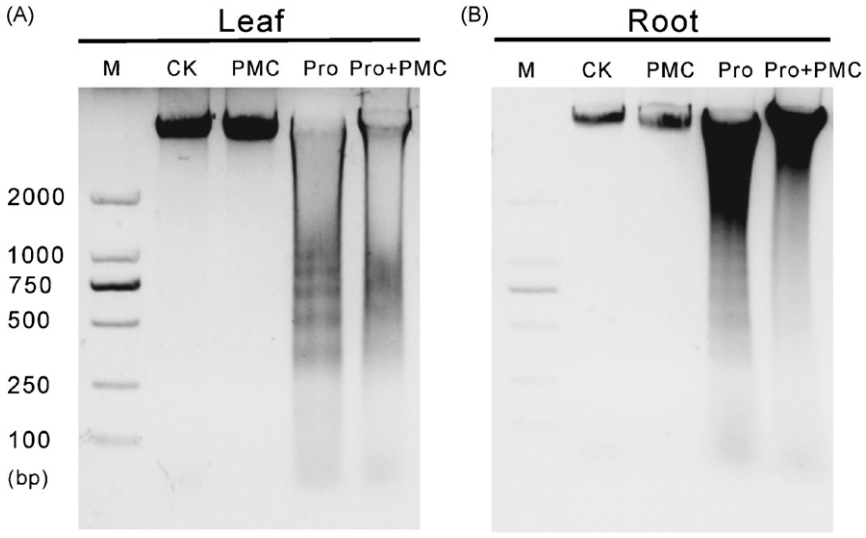

Fig. 6. Effects of pig manure compost (PMC) on the prometryne-induced DNA fragmentation. M: the molecular marker; CK: control (PMC-free); PMC: soil + 5% PMC; Pro: soil + prometryne (8 mg kg^{-1}), and Pro + PMC: 5% PMC + prometryne (8 mg kg^{-1}).

stimuli [42]. HO-1 is a ubiquitous enzyme catalyzing degradation of heme into biliverdin and releases free iron and carbon monoxide. Biliverdin is reduced to bilirubin by bilirubin reductase and considered as a potent antioxidant [43]. Our analysis showed up-regulation of *HO-1* expression with prometryne. Compared to prometryne treatment alone, addition of PMC to the soil reduced the transcript abundance of *HO-1* in leaves, but increased it in roots, although to less extent (Fig. 5).

3.6. Genotoxicity with prometryne in the presence of PMC

To support the molecular response of wheat to prometryne, we performed an additional experiment related to DNA degradation, a hallmark of cellular programmed cell death (PCD) in plants which is frequently used to monitor the molecular injuries [44]. Under environmental stimuli, ROS have been implicated in signaling chromatin condensation and DNA fragmentation. Normally, the PCD occurrence during the hypersensitive response (HR) is preceded by an oxidative burst that is mainly attributable to the activation of several enzymatic systems involved in ROS generation [45]. As stated in a preceding part of this study, prometryne triggered the ROS production in wheat leaves (Fig. 3). Therefore, it was possible that DNA fragment would occur. Treatment with 8 mg kg^{-1} prometryne triggered obvious DNA laddering in both leaves and roots (Fig. 6). However, the prometryne triggered DNA fragmentation was greatly reversed by PMC treatment. These results also suggest that PMC delivered the protection against prometryne injury by preventing DNA fragmentation.

4. Conclusion

To protect crops from weed and disease attack, the pesticide usage has been ever increasing. Owing to its toxic nature, pesticide contamination has become one of the serious public concerns, with respect to its possible impact on both ecosystem and human health. Recently, an increasing interest has been focused on the assessment of the consequence of exogenous organic carbon on the behavior of pesticides in soils. The organic matter serves as a primary amendment in sorption, transformation and mobility of many organic pollutants. The present study shows that PMC alleviated prometryne toxicity to wheat through a series of morphological and biochemical changes that were consistent with mitigation of genotoxicity and growth inhibition. Moreover, PMC prevented cellular toxic effects by reducing the prometryne accumulation in wheat. Although the precise mechanism is largely unknown, it is convincingly illustrated by our study that PMC amendment is able to mitigate the prometryne-induced toxicity and serve as a strategy for the rational usage of PMC in the agronomic and environmental management. Further detailed research will be required on the effect of PMC components (*e.g.* carbon or nitrogen amendment) on the bioavailability and degradation of the pesticide in the environments. Also, it is interesting to perform genome-wide analyses of transcriptome in model or genome-sequenced plant species under the toxic pesticides stress. Identification of pesticide stress-responsive will provide a basis to molecular breeding designed to improve the environmental stress tolerance to pesticide residues and phytoremediation of pesticide-contaminated soils.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhazmat.2010.08.041.

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