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Title: Uptake, translocation and possible biodegradation of the antidiabetic agent metformin by hydroponically grown *Typha latifolia*

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Abstract

The increasing load of pharmaceutical compounds has raised concerns about their potential residues in aquatic environments and ecotoxicity. Metformin (MET), a widely prescribed antidiabetic II medicine, has been detected in high concentration in sewage and in wastewater treatment effluents. An uptake and translocation study was carried out to assess the ultimate fate of MET in phytoremediation. MET was removed from media by *Typha latifolia*, and the removal processes followed first order kinetics. After 28 days, the removal efficiencies were in a range of 74.0±4.1~81.1±3.3%. In roots, MET concentration was increasing during the first two weeks of the experiment but thereafter decreasing. In contrast, MET concentration was continuously increasing in rhizomes and leaves. Bioaccumulation of MET in roots was much higher than in leaves and rhizomes. As degradation product of metformin in the plant, methylbiguanide (MBG) was detected whereas guanylurea was undetectable. Moreover, MBG concentration in roots was increasing with exposure time. An enzymatic degradation experiment showed the degradation rate followed the order of MET < MBG << guanylurea. This may explain the low concentration of MBG in plant. The findings of this study contribute to understand and evaluate the potential for phytoremediation of such kind of contaminants.

Keywords. metformin, phytoremediation, removal efficiency, methylbiguanide

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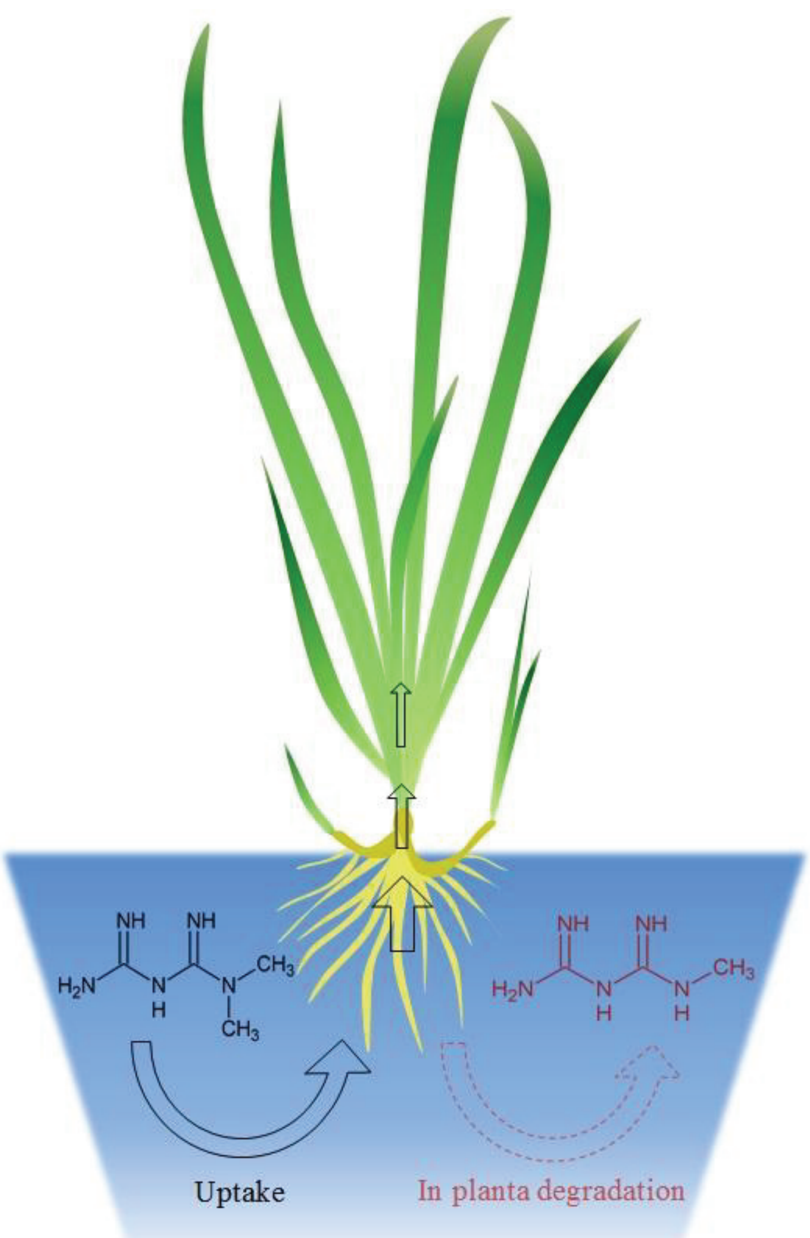
Graphical abstract



1. Introduction

Pharmaceuticals and personal care products (PPCPs) found in the environment have attracted increasing concern in the last decade [1,2]. Many pharmaceuticals are stable chemical entities which are not or only incompletely removed in wastewater treatment plants (WWTPs), and that is why many of them have been detected in WWTP effluents and even in our surface waters [3-5]. The eco-toxicological impact of pharmaceuticals is difficult to predict because of their trace-level concentrations (ranging from $ng \cdot L^{-1}$ to $\mu g \cdot L^{-1}$), potential persistence and biological activity in the aquatic environment [6,7].

Pharmaceuticals inducing environmental risk usually go along with high consumption volumes, but many of them are poorly investigated with regard to their environmental distribution and transport. The antidiabetic II medicine metformin (MET) is one of the most prescribed pharmaceuticals [8-10]. In Germany, MET usage almost tripled in the last 10 years to 1100 tons in 2010, and the trend towards increasing sales numbers grows continuously [11]. MET is metabolized in the human body only to a minor extent and excreted unchanged in the urine [12,13]. Therefore, it is not surprising to find this chemical in WWTPs and in surface water. Previous studies reported MET concentrations in a range of 1.2~118 $\mu g \cdot L^{-1}$ in WWTPs and 0.06~3.1$\mu g \cdot L^{-1}$ in surface water [5,11,14,15]. Although MET is potentially degraded in activated sludge to a dead-end metabolite, guanylurea, it can still be detected at high concentrations in effluent and surface water because of its high influent load [5].

Phytoremediation is an efficient technology to clean up a variety of organic and inorganic pollutants in soils and waters [16]. Plants and their associated microbes can be used for

phytoremediation in constructed wetlands, soils or hydroponic systems [17]. Phytoremediation can be a successful technology for removing PPCPs, which might be implemented as tertiary steps in traditional WWTPs at low operating and maintenance expenses [18,19]. Recent studies have shown that MET can be taken up by crops and vegetables followed by accumulation in leaves [20,21]. However, the final fate of MET in phytoremediation process and its potential removal from effluents is still poorly investigated.

The aims of the current study were: 1) to assess the removal efficiencies of MET from hydroponic solutions by *Typha latifolia* plants; 2) to evaluate the uptake and translocation of MET into roots, rhizomes and leaves; 3) to identify possible biodegradation products in plants.

These results may help to evaluate the environmental fate of MET in wetland plants as well as ti promote the further development and application of phytoremediation of similar compounds.

2. Materials and Methods

All chemicals and solvents used were of highest quality commercially available indicated in the Supplementary data (S, Table S1)

2.1 Plant Material

Typha latifolia.L plants were ordered from a local nursery (Jörg Petrowsky, Eschede, Germany) and the rhizomes were thoroughly washed with tap water. Plants were grown on perlite in 5L vessels and then transferred to a greenhouse with 12 h of light/12 h of darkness at 23/18°C and a humidity of 65 %. Nutrients were provided to plants by a modified

Hoagland nutrient solution (described in the Supplementary data section). Plants were acclimated to greenhouse conditions at least two months before the experiments.

2.2 Experiment setup

The pots were filled with 1.5 L perlite and each pot contained 1 L nutrient solution that was spiked with MET to reach concentrations of 50, 150 and 250 μmol·L^{-1} (corresponding to 6.5~32.3 mg·L^{-1}), respectively. For each of the three MET concentrations, three assays were set up corresponding to each exposure period studied, i.e., 1, 3, 7, 14 and 28 days. Control plants were grown under the same conditions, only in absence of MET. Furthermore, pots were set as controls (a) without plants but including perlite, and (b) without plants and perlite. In both sets, MET concentrations were not significantly decreasing during the experimental period. Three replicates were treated for each assay and all assays were performed simultaneously. For each exposure time, one assay was harvested. Root, leaf, rhizome and nutrient solutions were collected, frozen and stored, respectively. Additional details for determination of MET and its biodegradation products in plants are described in the Supplementary data.

2.3 Biodegradation in Plant Tissue Enzyme Extracts

Crude enzyme extracts of *T. latifolia* roots, rhizomes and leaves were prepared by pestling aliquots of sheared plant tissue under liqu. N_2 and adding 50 mM potassium phosphate buffer (pH 7.0) to the frozen powder. 50 μL stock solution of MET, methylbiguanide (MBG) and guanylurea were added to 950 μL crude enzyme extract to yield a final concentration to 250 μM of each compound, respectively. The reaction solution was mixed and incubated at 25 °C

for 60 min, and then quenched by adding 50 µL glacial acetic acid. The resulting solutions were analysed by LC-MS/MS. Additional details are provided in the Supplementary data.

2.4 Analysis for Metformin and its Biodegradation Products by LC−MS/MS

The analysis of water and plant tissue extracts was carried out by solid phase extraction followed by LC-MS/MS. The HPLC system (Varian ProStar 210, Darmstadt, Germany) was coupled to an ion trap mass spectrometer (Varian 500-MS, Darmstadt, Germany) with an electrospray interface operated in the positive ion mode. Separation was achieved using a Synergi Polar-RP 80a column (150 mm × 2 mm, 4 µm, Bischoff, Germany) at a flow rate of 0.3 ml·min^{-1}. Further details are available in the Supplementary data.

3. Result and Discussion

3.1 Removal of MET from Nutrient Solution

MET was continuously removed from the nutrient solution by *T. latifolia* during the whole exposure time. By the end of the experiment, the maximum removal efficiency ranged from 74.0±4.1% to 81.1±3.3% for the initial concentrations of 250 µmol·L^{-1} and 50 µmol·L^{-1}, respectively (Figure 1a). MET was rapidly removed from the nutrient solution during the initial 7-day period, with removal efficiencies in the range of 39.9%~58.9%. The removal processes conformed well to first-order kinetics at rate constants of 0.0969, 0.0655 and 0.0631 day^{-1} for the concentrations of 50, 150 and 250 µmol·L^{-1}, respectively (Figure 1b). In the present study, MET removal efficiencies were significantly related to the exposure time

(Pearson correlation coefficient 0.859~0.931, $p<0.01$). Furthermore, the initial MET concentrations and the MET removal efficiencies clearly fit with a linear relationship at each corresponding exposure time (in all cases, the R2 value was in a range of 0.950~0.999).

Variations of MET concentration in the nutrient solution were negligible in controls during the experimental period. Abiotic processes such as photodegradation and adsorption to the walls of plastic vessels seem to play only a minor role in MET removal. In addition, MET as a hydrophilic compound cannot be expected to adsorb on the lipophilic surfaces of roots and rhizomes. Therefore, MET removal from the nutrient solution is expected to depend to a high degree on the uptake by plants.

The removal processes of MET followed first order kinetics. Zhang et al. [22] studied the removal of five different pharmaceuticals by *Scirpus validus* and reported rate constants in a range of 0.023~0.403 day^{-1}. However, the removal process includes different mechanisms such as adsorption, uptake and transformation. Thus, the rate constants should only be considered as a phenomenological value [23]. MET was removed more efficiently from nutrient solutions at lower treatment concentration than at higher treatment concentration. This result is in good agreement with Dordio et al. [24] who observed the same phenomenon in the uptake of carbamazepine by *Typha spp*. Many studies show that the initial concentration of pharmaceuticals and the removal efficiencies clearly fit a linear relationship [24,25].

3.2 Accumulation of MET in Roots

In order to quantify the accumulation of MET in plants, the pharmaceutical was scanned in roots, rhizomes and shoots of *T. latifolia*. In fact, MET was detected in all *T. latifolia* tissues of all treated concentration levels. In roots, MET concentrations increased with exposure time up to day 7 in the 50 $\mu mol \cdot L^{-1}$ treatment, but decreased thereafter. For 150 and 250 $\mu mol \cdot L^{-1}$ treatments, MET concentrations in roots started to decrease after day 14 (Figure 2a). A maximum 11.32 $\mu mol \cdot g^{-1}$ (FW) of MET was found in plants exposed to 250 $\mu mol \cdot L^{-1}$ treatment after 14 days of exposure. Statistical analysis showed a significant positive correlation between MET concentration in roots and initial concentration in nutrient solution at each time point (Table S2). The bioaccumulation factors (BAFs) are defined as the ratio of MET concentrations in plant tissues to MET concentrations in the nutrient solutions. Table 1 shows the BAFs for roots, ranging from 8.37 to 53.34.

Many uptake processes of xenobiotics are governed by the physico-chemical properties of the contaminant, chemical speciation, and the plant itself. Chemicals of high hydrophilicity (log P < 0.5) are not sufficiently adsorbed to roots but may be actively transported through plant membranes [26, 27]. Surprisingly, our results demonstrate high MET concentrations and BAFs for roots. This result is also noted by Herklotz and coworkers [28] who find that Salbutamol (log P=0.64) exhibits high bioaccumulation (BAF=9.048) in cabbage roots. Apparently, MET can be taken up by crops and vegetables from soil while exhibiting a generally high bioaccumulation in roots (BAF=2~10) [20,21]. By comparison, our results showed higher BAFs than the previous studies in roots. This is probably caused by a higher bioavailability of the compound in the hydroponic systems than in soil systems.

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Plant uptake includes both, the apoplastic and the symplastic pathway. The apoplastic pathway is interrupted at the endodermis by the Casparian strip. The casparian strip is a band of cell wall material consisting of suberin and lignin, deposited on the radial and transverse walls of the endodermis to block uncontrolled passive movement of water and chemicals [29]. At this point, the apoplastic flow is forced to move into the symplast pathways, or to cease. Ionizable compounds have low potential for passive diffusion through lipophilic biomembranes. Thus, such compounds are not expected to enter roots at a high rate. However, as previously observed, a surprisingly high uptake and translocation of MET to oily rape seeds indicates that some active processes might be involved in the uptake of such a polar and dicationic compound [21]. Our previous study assumed that organic cation transporters may play an important role in the uptake and transport process of MET [30]. Thus, the above mentioned mechanism for the uptake of chemicals by roots may be of general importance for non-ionised compounds [26].

Uptake of MET into the roots was quite rapid and the uptake rates ranged from 0.296 $\mu mol \cdot g^{-1} \cdot day^{-1}$ (50 $\mu mol \cdot L^{-1}$ treatment) to 1.461 $\mu mol \cdot g^{-1} \cdot day^{-1}$ (250 $\mu mol \cdot L^{-1}$ treatment) up to day 7 (Table S3). This result is in agreement with earlier studies demonstrating that xenobiotics can be taken up by plants within a short time [31,32]. On principle, there should be a balance between uptake and degradation of MET. At the beginning of the experiments, uptake rates were much higher than degradation rates. With time, uptake rates decreased while degradation rates increased, probably because MET concentration decreased in media and MET concentration increased in roots at the same time. MET concentration in roots

continuously increased to the maximum critical value when the uptake rates were equal to the degradation rates, and decreased thereafter.

3.3 Translocation of MET from Roots to Rhizomes and Leaves

MET concentrations were in a range from 0.011 to 1.039 $\mu mol \cdot g^{-1}$ (fresh weight) in the rhizomes at day 28 (Figure 2b), as well as 0.010 to 0.305 $\mu mol \cdot g^{-1}$ (fresh weight) in the leaves (Figure 2c). MET concentrations in both, rhizomes and leaves increased with exposure time (except 50 $\mu mol \cdot L^{-1}$ treatment for rhizomes). Compared to roots, MET concentrations were relatively low in rhizomes and leaves. After 28 days of exposure, we found the highest BAF values of 4.40 for rhizomes in 250 $\mu mol \cdot L^{-1}$ treatment and 1.39 for leaves in 50 $\mu mol \cdot L^{-1}$ treatment (Table 1). Statistical analysis yielded a significantly positive correlation between MET concentration in rhizomes or leaves and the initial concentration in the nutrient solution at each time point (Table S3).

MET concentrations were relatively low in rhizomes and leaves. Similar results were reported for carrot and barely by Eggen and coworkers [20]. Uptake of other pharmaceuticals has also been previously reported [22,24,28,33]. Our earlier study confirmed that the transport processes of ionic and non-ionic compounds were different. Since ionic compounds can only cross membranes by active transport, the activities of potential transport proteins for these compounds may have more significance than the log P value [30]. Another study also indicated that the activity of carrier proteins were necessary for transport of pharmaceuticals by plants [28].

Generally, compounds with an optimum hydrophobicity (log P=0.5~3) are preferred to be taken up and translocated by plants [16,26]. Recent studies indicate that even more hydrophilic chemicals can also have high potentiality of uptake and translocation [34]. However, the present study shows that MET can be taken up by roots at high levels but is poorly translocated to leaves, due to a variety of possible factors including plant species, size, growth conditions, metabolism or volatilization. Furthermore, other studies found plant uptake of xenobiotic organic compounds was independent of hydrophilicity [35,36]. These results suggest that log P may have limited predictive value for uptake and translocation of xenobiotic contaminants by plants.

Plant uptake of neutral compounds has been frequently reported. However, uptake of ionic compounds which included numerous uncertainties has been studied to a much lower extent [37]. Uptake and translocation of ionisable compounds mainly depend on the chemical´s pKa, the solution pH value and the permeability ratio between neutral and ionic molecules. Since MET is present as dicationic molecule in media (pH~6), it was more difficult to predict its environmental fate in these studies [38]. Although the spiked MET concentration was significantly higher than its typical environmental concentrations, it was chosen to allow assessment of the mechanisms involved in plant uptake and biodegradation. However, a hydroponic system may be relatively limited to predict the fate of contaminants in actual engineered conditions or in groundwater. There, reasons for lower bioavailability might include sorption to soil matrix [39]. Therefore, further investigation is still needed, especially in the field.

3.4 Identification of the Possible Biodegradation Products in Plant

MBG concentrations increased with the exposure time at all treated concentrations in roots, and a maximum 26.7 $nmol \cdot g^{-1}$ (fresh weight) of MBG concentration was found in 250 $\mu mol \cdot L^{-1}$ treatment after 28 days exposure (Figure 3). We also detected MBG in rhizomes and leaves, but only after 28 days exposure and concentrations were too low to quantitate. Statistical analysis showed a significant positive correlation between MBG concentration in roots and the initial concentration in nutrient solution at each time point (Table S3). In addition, MBG was not detected in water samples, which supports that biodegradation is an in-planta process.

To our knowledge, MBG has never been reported as a biodegradation product of MET in plants. Collin et al [40] described MBG as one of the primary hydroxyl free radical-induced oxidation products of MET in aqueous solution. N-dealkylation is a frequently found metabolic reaction of xenobiotics in plants [41]. Kawata et al [42] found that the hepatic microsomal cytochrome P-450 oxidase system could also catalyze N-demethylation of methylguanidine to guanidine in humans. Trautwein and Kümmerer [43] suggested dealkylation as the mechanism for degradation of MET by microorganisms. However, whether a similar mechanism also exists in plants is still unknown.

Up to now, little knowledge has been collected about in planta biodegradation products of MET. Guanylurea, a stable dead-end metabolite of MET was reported previously in activated sludge [43]. Eggen and coworkers [21] detected guanylurea as a MET related biodegradation product in barley grains, bean pods and potato peel, but not in leaves, rhizomes and roots. However, guanylurea has not been detected, neither in water nor in plant tissues, in our studies. Furthermore, recent studies indicate that guanylurea can still be degraded to

guanidine and ammonia rapidly [44]. Under these conditions, guanidine can be oxidized to urea and later hydrolyzed to ammonia [45].

Adsorption and photodegradation did not play important roles in MET removal as only 4.2%~6.6% of MET was eliminated from nutrient media in the control. However, a percentage in a range of 33%~48% of initial MET concentrations was found in plants at the end of experiment, which was less than the total amount removed from nutrient solutions (58%~74%). Since MBG can only contribute a small part of MET mass loss, this indicates that either MBG was not the only dead-end biodegradation product of MET or further degradation of MBG would lead to minor, not detected metabolites.

3.5 Enzymatic degradation experiment

The degradation of selected compounds (MET, MBG and guanylurea) was assessed in crude enzyme extracts from *T. latifolia* roots, rhizomes and leaves, respectively. In comparison to MET and MBG, guanylurea concentrations significantly decreased after 20 min (Figure 4, S4,5). The highest degradation rates of MET, MBG and guanylurea were 0.061, 0.108 and 0.256 μmol·mg protein^{-1}·min^{-1}, respectively. The degradation rate is represented by the slope of a linear fit of a degrading process (0-20 min). In all three enzyme extracts, the degradation rates of MET and MBG were much lower than guanylurea (Table S4).

Michaelis-Menten kinetics is a general model to describe the enzymatic reactions, but direct comparison of Michaelis-Menten constants (*Km*) for enzymes within a crude extract from plant is not reliable [46]. Therefore, we did not fit and calculate the *Km* value of the present experimental data. However, previous studies found the *Km* value of N-demethylation was

much higher than N-hydroxylation in hepatic microsomal catalytic degradation of guanidines [42,47]. Since a high Km indicates low affinity, it had been hypothesized that N-demethylation of MET is much more difficult than N-hydroxylation, and N-demethylation could be the rate-limiting step for the degradation of MET in plant. Therefore, it is possible that the degradation rates could be improved by N-demethylation of MET, and after removal of two methyl groups, biguanide could be rapidly degraded even if present in minimal concentration. This could be an explanation for the unanticipated results that we did not detect guanylurea in any of the plant tissues. In addition, previous studies also showed that the N-demethylation of pharmaceuticals and herbicides may be mediated by the same microsomal cytochrome P450 fraction in plants [48, 49].

4. Conclusions

This study investigated the fate of MET in plants used for phytoremediation. The results show that removal processes followed first order kinetics. MET can be taken up from medium, but the translocation via roots to shoots was restricted. Thus, a simply passive diffusion modeling may be not applicable for the ionic compounds due to numerous uncertainties. MBG was first detected as a biodegradation product of MET in plant, and yet its concentration was low. It is important to study the fate of MET and its biodegradation products in plant-based systems. This will help to improve our understanding of phytoremediation for PPCPs. Since plants are the base of the food chain, our finding may also contribute to the fact that root vegetables could impose a higher exposure risk for human than leafy vegetables when from a xenobiotic

contaminated environment, e.g. after sewage sludge application. In addition, to identify the possible biodegradation pathways needs further studies.

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Figures

1. The removal efficiencies of MET by *T. latifolia* at different exposure time (a); the first-order kinetics fitting of removal process (b). Error bars indicate SD (n=3).
2. Concentrations of MET in different tissues of exposed *T.latifolia*. (a) roots; (b) rhizomes; (c) leaves. Error bars indicate SD (n=3).
3. Concentrations of MBG in roots of exposed *T.latifolia*. Error bars indicate SD (n=3).
4. Concentrations of the selected compounds during exposure to root enzyme extracts. Error bars indicate SD (n=3). 1mL enzyme extracts was from 0.03g plant material FW. Significant analysis without controls, **p<0.01; ***p<0.001.

Tables

1. Bioconcentration factors of MET in roots, rhizomes and leaves of exposed *T. latifolia*

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Figure 1a

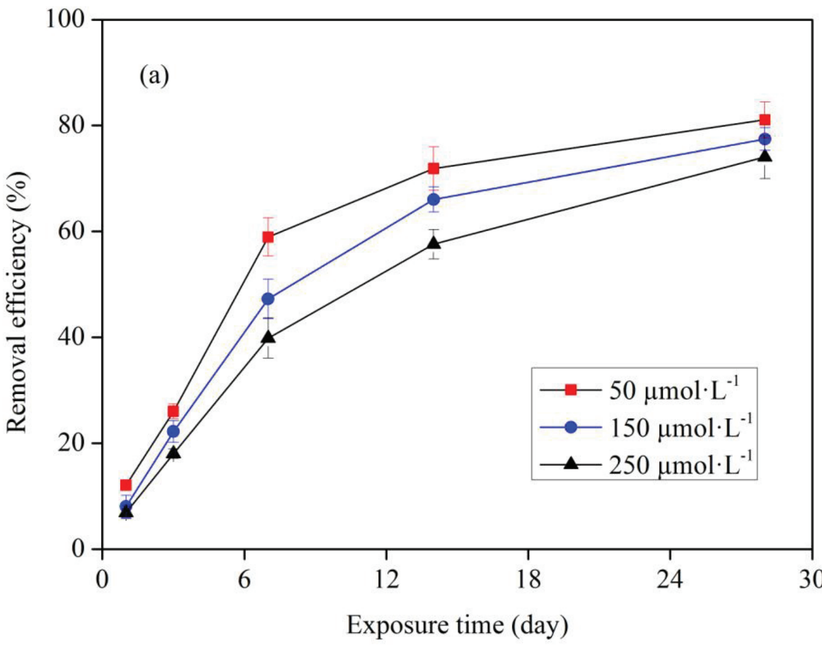

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Figure 1b

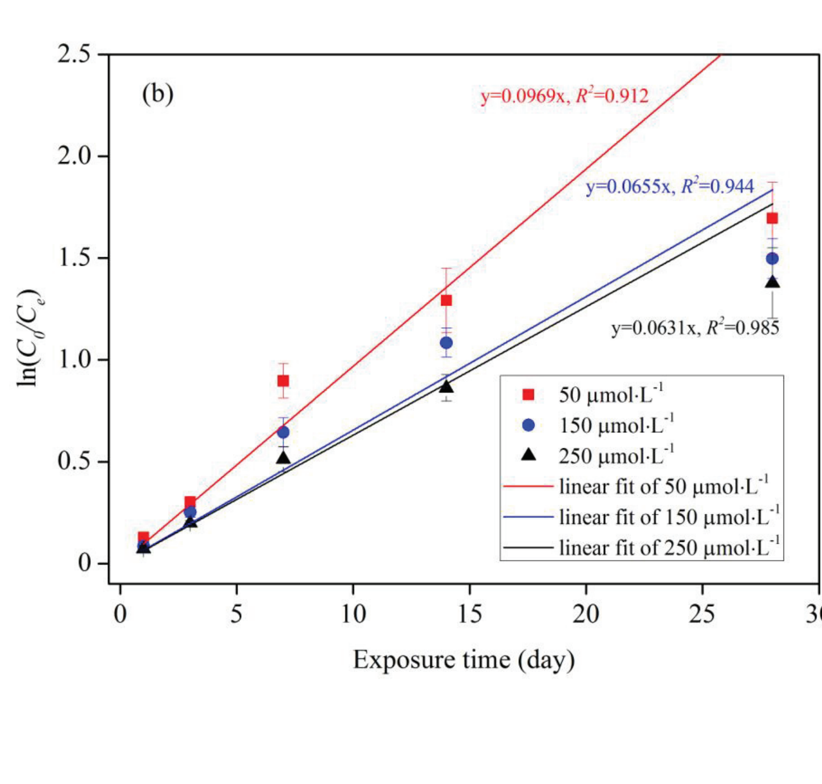

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Figure 2a

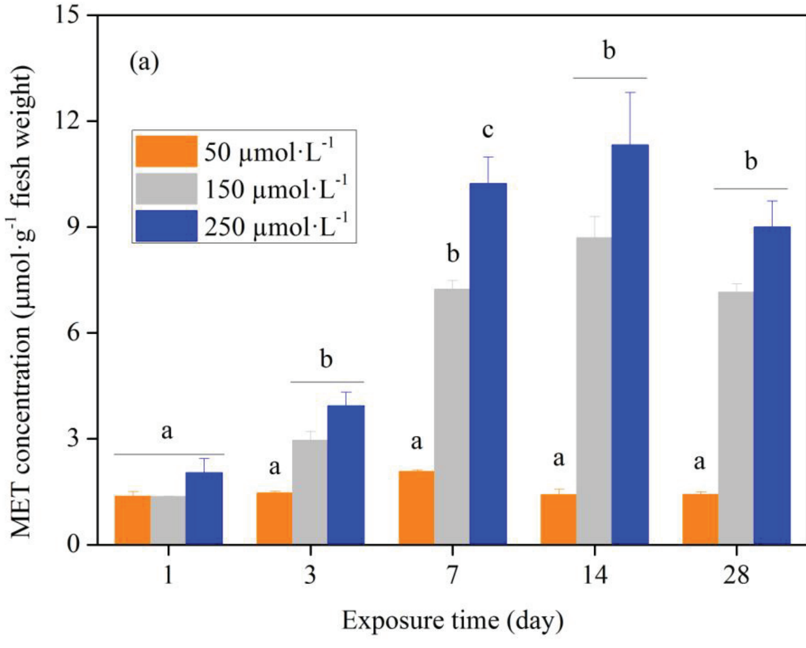

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Figure 2b

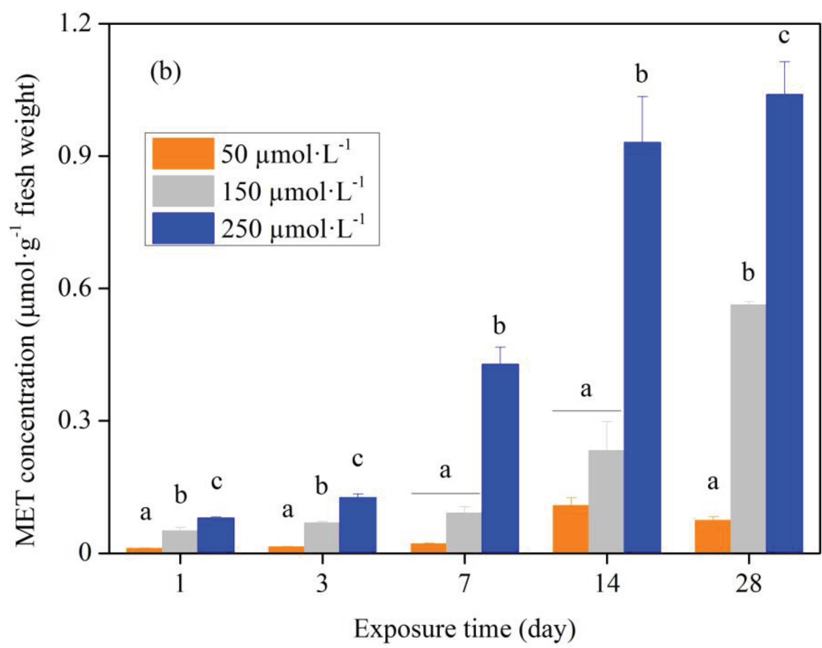

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Figure 2c

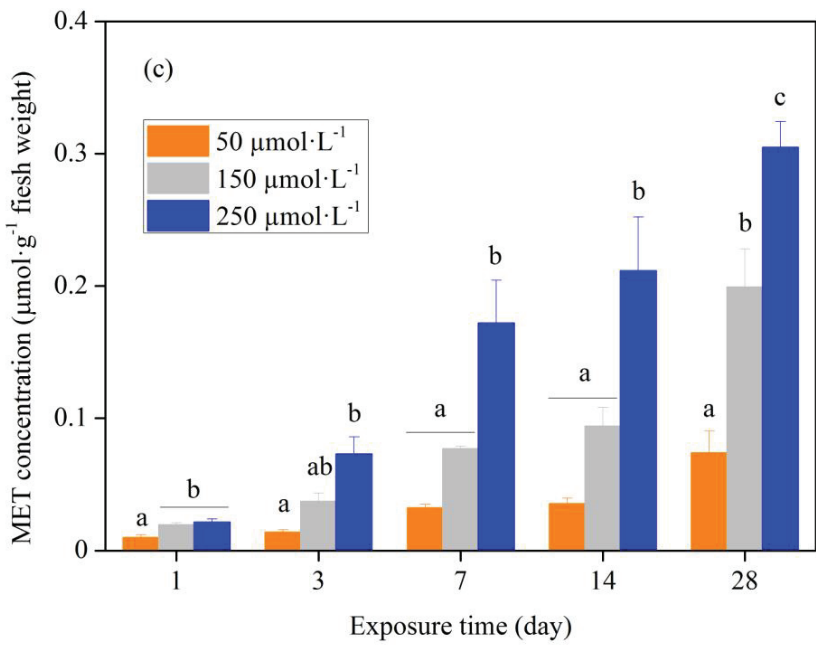

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Figure 3

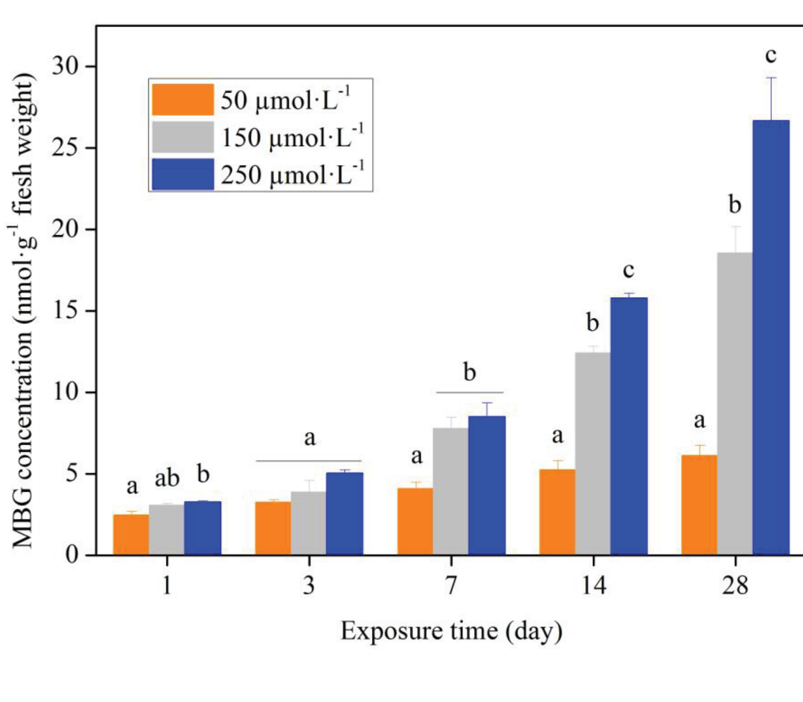

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Figure 4

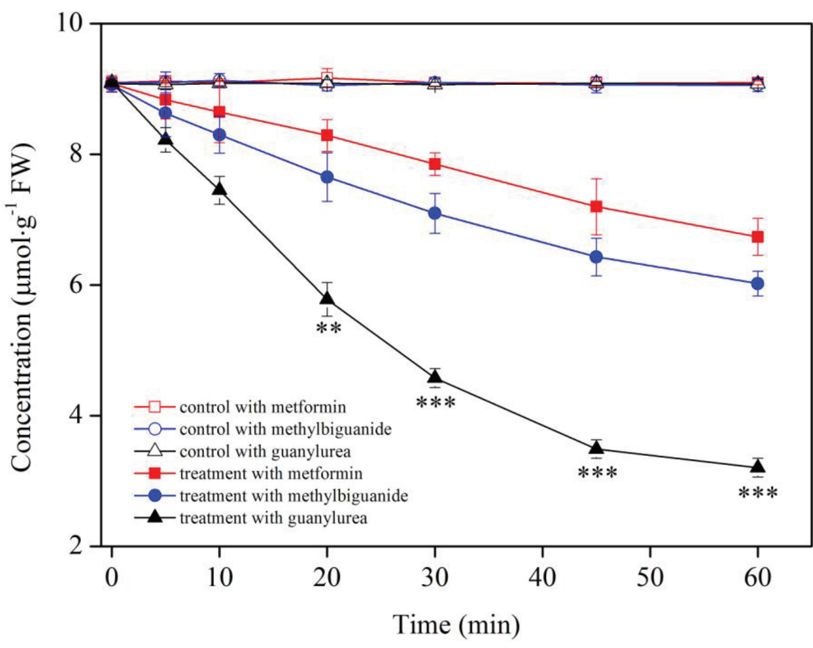

Table 1

Spiked initial concentrations (μmol·L^{-1})	1day	3days	7days	14days	28days
Roots					
50	25.46±1.94	27.44±1.50	38.75±0.96	26.42±3.26	26.60±0.97
150	8.37±0.34	18.04±0.69	44.70±3.30	53.34±2.19	44.13±3.05
250	8.61±1.65	16.65±1.62	43.23±2.93	47.99±6.62	38.06±3.02
Rhizomes					
50	0.20±0.02	0.26±0.01	0.38±0.05	2.00±0.27	1.38±0.15
150	0.31±0.05	0.42±0.01	0.56±0.09	1.46±0.43	3.47±0.20
250	0.33±0.01	0.53±0.04	1.81±0.16	3.94±0.47	4.40±0.34
Leaves					
50	0.18±0.04	0.26±0.03	0.61±0.07	0.66±0.06	1.39±0.32
150	0.12±0.01	0.23±0.03	0.48±0.03	0.59±0.12	1.25±0.24
250	0.09±0.01	0.31±0.05	0.73±0.14	0.90±0.09	1.29±0.09

Supplementary data to the Article:

Title: Uptake, translocation and possible biodegradation of antidiabetic agent metformin by hydroponically grown *Typha latifolia*

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Contents

1. Experimental methods, pg. S3
2. Analytical method of LC-MS/MS, pg. S6
3. Pearson correlation analysis, pg. S11
4. Uptake rates, pg. S12
5. Enzyme extract data, pg. S13
6. Degradation rates, pg. S15

1. Experimental methods

Chemicals. Metformin HCl (MET), methylbiguanide HCl (MBG) and guanylurea are produced by Sigma-Aldrich (Germany). Solvents were LC-MS grade and obtained from Roth (Germany). Ultrapure water was prepared in a Milli-Q water purification system. Inorganic nutrients for modified Hoagland solutions, and all other chemicals were analytical grade.

Nutrients. A modified Hoagland nutrient solution was prepared with the following chemical composition: 2.5 mmol·L^{-1} K^+, 2.0 mmol·L^{-1} Mg^{2+}, 2.0 mmol·L^{-1} Ca^{2+}, 2.0 mmol·L^{-1} SO_4^{2-} , 6.0 mmol·L^{-1} NO_3^-, 0.5 mmol·L^{-1} $H_2PO_4^-$,50 μmol·L^{-1} Fe^{2+}, 50 μmol·L^{-1} BO_3^{3-}, 1μmol·L^{-1} Mn^{2+}, 0.5 μmol·L^{-1} Cu^{2+}, 0.5 μmol·L^{-1} Zn^{2+}, 0.1 μmol·L^{-1} MoO_4^{2-} and the pH was adjusted to 6.0.

Experiment Setup. Plants of uniform size were selected for the experiments. Plant roots were rinsed in distilled water and then transferred to 2.5 L pots which contained 1.5 L perlite. Each pot was supplied with 1 L nutrient solution that was spiked with MET at concentrations of 50, 150 and 250 μmol·L^{-1}. The blank plants were grown under the same condition, only in absence of MET. Plants were acclimated to greenhouse conditions at least two months before use. Then all pots, except the controls, received equivalent amounts of MET. For each of the three MET concentrations, three parallel treatments were set up corresponding to each exposure period studied, i.e., 1, 3, 7, 14 and 28 days. Three replicates were treated for each assay and all assays were performed during the same period of time. Additionally, three replicates were set up for the control throughout the experimental period. For each exposure time, one replicate was harvested. Root, leaf and rhizome were collected respectively at the

same time, frozen in liquid nitrogen and stored at -80°C. The nutrient solutions remaining in each pot were analysed at each exposure period.

Biodegradation in Plant Tissue Enzyme Extracts. A method for crude enzyme extracts has already been described in the previous study [1]. In short, to prepare crude enzyme extracts of *T. latifolia* root, rhizome and leaf tissues, 1 g tissue material were ground in liquid nitrogen, then dissolved and stirred in 50 mM potassium phosphate buffer (pH 7.0) for 30 minutes on ice. The homogenate was centrifuged at 15250 g for 30 minutes at 4°C. 5 mM MET, MBG and guanylurea stock solution were also prepared in 50 mM potassium phosphate buffer (pH 7.0). 50 µL stock solution of MET, MBG or guanylurea was added to 950 µL crude enzyme extract to yield a final concentration to 250 µM of each compound, respectively. The reaction solution was mixed and incubated at 25 °C in a water bath for 60 min, then reactions were quenched by adding 50 µL glacial acetic acid. The resulting solutions were analysed by LC-MS/MS. Concentration of proteins in the crude enzyme extract (0.018-0.105 $mg \cdot mL^{-1}$) was measured using the Bradford assay [2].

Table S1. Structure and physicochemical properties of chemicals used in this study

chemicals	structure	molecular weight	pKa	Log D (pH at 7.4)	Log P
Metformin		129.16	12.33 and 10.27[a]	-4.30[b]	-2.31[b]; -2.64[c]
Methylbiguanide		115.14	11.75 and 9.69[a]	-3.14[b]	-1.30[b]; -1.53[c]
Guanylurea		102.10	13.62 and 5.81[a]	-1.82[b]	-1.89[b]; -3.57[c]

[a]ChemAxon, [b]ACD/Labs, [c]EPISuite™

2. Analytical method of LC-MS/MS

Plant Tissue Extracts. The analysis for determination of MET in plants followed a recently published method [3]. Briefly, 0.2 g fresh plant material was extracted with 1.8 ml extraction solution of ammonium acetate/formic acid. The mixture was vortexed, ultrasonicated and centrifuged, the supernatant was filtrated (0.45 μm, nylon) then transferred to a solid phase extraction (SPE) column (Bond Elut LMS, Agilent, Germany), eluted and injected into the LC-MS/MS system. The calibration for extraction processes (including SPE) were performed by use of blank plant tissue spiked with MET and the recovery was > 90%.

LC-MS/MS Condition. The HPLC system (Varian ProStar 210, Darmstadt, Germany) was coupled to an ion trap mass spectrometer (Varian 500-MS, Darmstadt, Germany) with an electrospray interface operated in the positive ion mode. Separation was achieved using a Synergi Polar-RP 80a column (150 mm × 2 mm, 4 μm, Bischoff, Germany) with a flow rate of 0.3 $ml \cdot min^{-1}$, and injection volume was 15μL. Eluent A was 0.1% formic acid in water and eluent B was 0.1% formic acid in acetonitrile. The gradient started with 97% of eluent A, after 2.5min it was decreased to 5% within 5 min, held for 2.5 min then increasing the percentage of eluent A back to the initial conditions within 1 min and held for 2min before the end. For MET determination, the precursor ion *m/z* 130.2 (M+H) giving product ions 113.2 and 71.2 was used for quantification and confirmation; for MBG determination, the precursor ion *m/z* 116.2 (M+H) giving product ions 60.2 and 74.2 was used for quantification and confirmation.

Reference

1. M.L. Card, J.L. Schnoor, Y.P. Chin, Transformation of natural and synthetic estrogens by maize seedlings. Environ Sci Technol. 47 (2013) 5101-5108.

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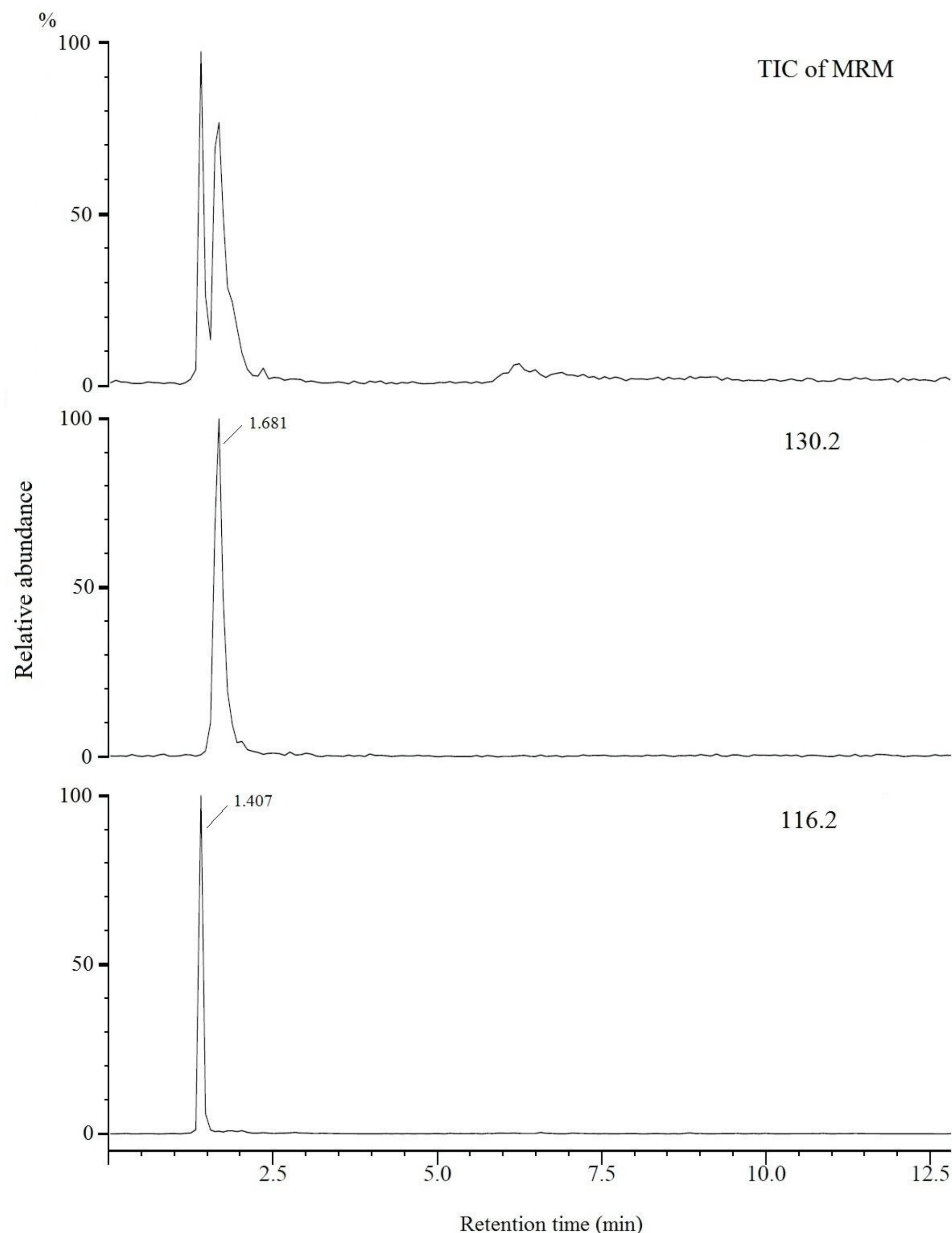

Figure S1. LC-MS/MS chromatograms of 1 μmol·L^{-1} standard sample (a mixture of metformin (m/z 130.2) and methylbiguanide (m/z 116.2)) prepared in extraction solution

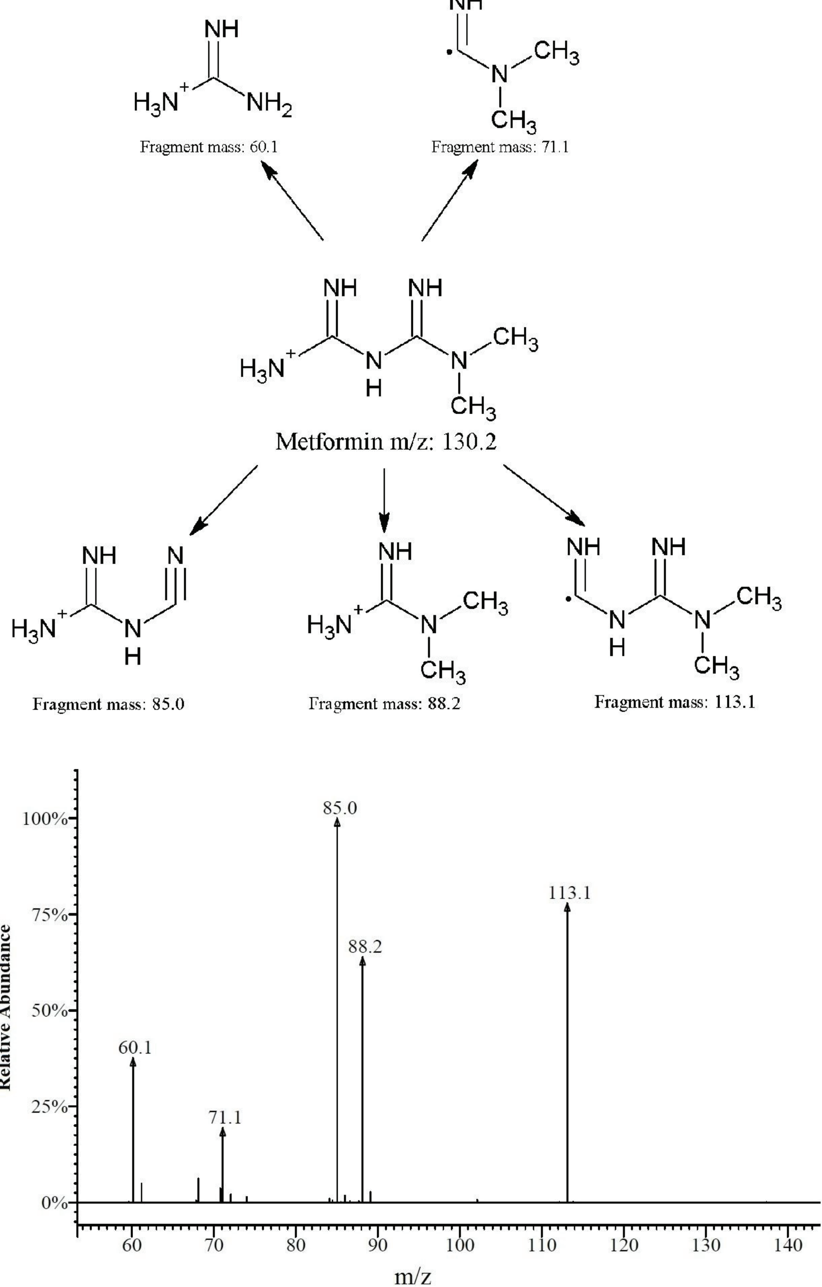

Figure S2. Product ion spectrum of metformin and its proposed fragmentation (MS/MS)

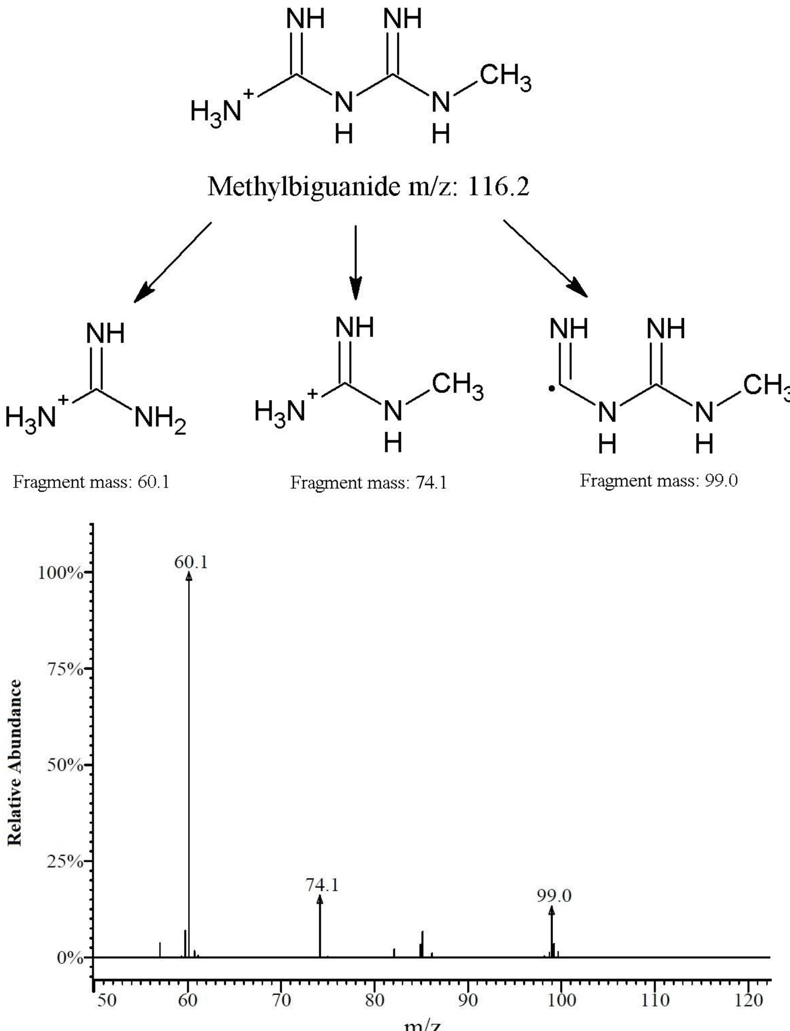

Figure S3. Product ion spectrum of methylbiguanide and its proposed fragmentation (MS/MS)

3. Pearson correlation analysis

Table S2. Metformin and methylbiguanidin concentration in different plant tissues at each time exposure points were analyzed by Pearson correlation (Pearson's coefficient).

Time (days)	MET			MBG
	roots	rhizomes	leaves	roots
1	0.581*	0.964**	0.893**	0.808**
3	0.930**	0.985**	0.806**	0.752**
7	0.970**	0.919**	0.891**	0.837**
14	0.920**	0.893**	0.885**	0.969**
28	0.941**	0.988**	0.948**	0.949**

*$p<0.05$; **$p<0.01$

4. Uptake rates

Table S3. Uptake rates of different plant tissue ($\mu mol \cdot g^{-1} \cdot day^{-1}$)

Time (day)	Root			Rhizome			Leaf		
	50	150	250	50	150	250	50	150	250
1	1.366±0.137	1.358±0.018	2.038±0.404	0.011±0.001	0.050±0.009	0.079±0.003	0.010±0.002	0.019±0.002	0.022±0.002
3	0.488±0.016	0.983±0.085	1.311±0.129	0.005±0.000	0.023±0.001	0.042±0.003	0.005±0.001	0.012±0.002	0.024±0.004
7	0.296±0.007	1.033±0.035	1.461±0.109	0.003±0.000	0.013±0.002	0.061±0.006	0.005±0.000	0.011±0.000	0.025±0.005
14	0.101±0.011	0.621±0.043	0.809±0.107	0.008±0.001	0.017±0.005	0.066±0.007	0.003±0.000	0.007±0.001	0.015±0.003
28	0.051±0.003	0.255±0.009	0.321±0.026	0.003±0.000	0.020±0.000	0.037±0.003	0.003±0.001	0.007±0.001	0.011±0.001

5. Enzyme extract data

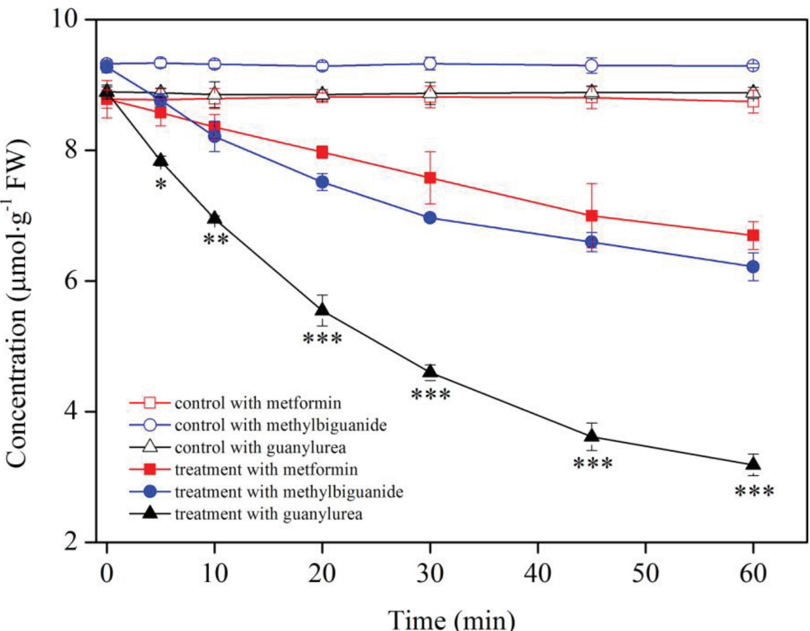

Figure S4. The selected compounds concentrations during of exposure to rhizome enzyme extracts. Error bars indicate SD (n=3). 1mL enzyme extracts was from 0.03g plant material FW. Significant analysis without controls, *p<0.05; **p<0.01; ***p<0.001.

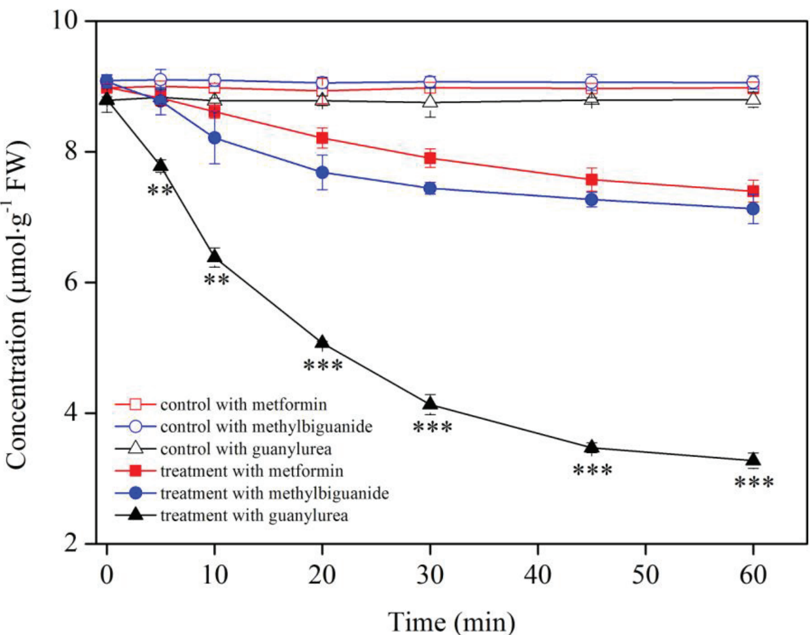

Figure S5. The selected compounds concentrations during of exposure to leaf enzyme extracts. Error bars indicate SD (n=3). 1mL enzyme extracts was from 0.03g plant material FW. Significant analysis without controls, **p<0.01; ***p<0.001.

6. Degradation rates

Table S4. The degradation rates (μmol·mg $protein^{-1}$·min^{-1}) of selected compounds in crude enzyme extracts

	metformin	methylbiguanide	guanylurea
root	0.061±0.025	0.108±0.015	0.256±0.019
rhizome	0.011±0.003	0.024±0.003	0.045±0.003
leaf	0.021±0.005	0.038±0.010	0.101±0.001