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EFFECT OF FOLIAR APPLICATION OF L-METHIONINE ON PHOTOSYNTHETIC, BIOCHEMICAL AND GROWTH ATTRIBUTES OF WHEAT AT TWO LEVELS OF NITROGEN

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ABSTRACT

The influence of L-Methionine (L-MET under field conditions revealed that nitrogen availability influenced ethylene production, leaf tissue nitrogen concentration, photosynthetic attributes, and growth in wheat. Ethylene production in the control (75 mg of N kg^{-1} soil) was two times greater than the treatment with high N concentration (150 mg N kg^{-1} soil). All the parameters showed the maximum increase with 20 mg L-MET kg^{-1} soil. The results are suggestive of the fact that the application of L-MET greatly increased relative growth rate (RGR), total leaf area, net photosynthesis and leaf tissue N concentration, depending on the concentration of soil available nitrogen.

Key Words: ethylene, photosynthesis, nitrate reductase, RGR, wheat.

INTRODUCTION

L-Methionine is a documented source of plant growth regulator ethylene, which plays fundamental role in various aspects of growth and photosynthesis (Pierik *et al.*, 2006; Ahmad *et al*,. 2012). Ethylene is to inhibit growth and create senescence in plant tissue (Abeles *et al.*, 1992), but recent studies revealed that it acts as growth stimulator at low concentrations (Fiorani *et al.*, 2002). Khan *et al.*, (2007) reported that it improved the photosynthetic attributes (stomatal conductance and greenness), relative growth rate and nitrate reductase activity in *Brassica juncea*. The effects of ethylene vary depending on its rate of production and the sensitivity or in-sensitivity of plant species (Pierik *et al.* 2006). A low rate of ethylene resulted in increased rate of photosynthesis (Khan *et al*., 2007) and leaf growth (Khan 2005). However, in contrast, higher ethylene concentration decreased leaf area (Khan 2005) and photosynthesis (Khan 2004). Nitrogen (N) is one of the major plant nutrients and plays an important role in photosynthesis and growth because it is essential in cell division and expansion, as it is a constituent of chlorophyll, thylakoid proteins and enzymes of the photosynthetic carbon reduction cycle (Marschner 1995; Khan *et al.*, 2007). Lynch and Brown (1997) reported that N deficiency was related to ethylene production and sensitivity.

Several studies have been reported about the effects of ethylene on growth and photosynthesis of wheat. However, the role of N availability on ethylene evolution and changes in relative growth rate, photosynthesis, and N accumulation has not been reported extensively. The effect of nitrogen availability on the production of ethylene, relative growth rate, net photosynthesis, and leaf tissue N concentration were examined.

MATERIALS AND METHODS

Wheat seeds (*Triticum aestivum* L.) cv. Sehr-06, were sown in 5 × 5 m plots at the experimental field in Faisalabad, Pakistan. The soil was a silt loam. Soil samples were collected from the experimental plots (30 cm). These samples were air dried, grinded and sieved through a 2 mm mesh. Available NO_3–N, phosphorus, and potassium were determined by procedures given in Handbook 60, USDA. Seeds of wheat were sown in the plots on November 15, 2008 and nitrogen was applied at a basal rate of 75 ppm (low N) and 150 ppm (high N) in the form of urea. After seedlings were established, 10 plants per m^2 were maintained. A precursor of ethylene (L-MET) was applied at 0, 10, 20 and 30 mg kg^{-1} soil (Fig.1), at anthesis (60 days after sowing). The treatments were arranged in a randomized complete block design

(RCBD) and replicated four times. 30 days after the application of L-MET, i.e. 90 days after sowing (DAS), the production of ethylene, plant growth characteristics, physiological parameters and leaf tissue N concentration were recorded from flag leaves.

Ethylene Production

Leaf materials were cut into small pieces and were placed into 30 mL tubes lined with moist paper to reduce evaporation from the tissue. One mL gas sample was taken from the tubes with a hypodermic syringe and assayed on a gas chromatograph (Carlo-Erba FVS-2300) run iso-thermically and in a capillary column packed with Porapak N. The following conditions were maintained in the gas chromatograph: carrier gas, N_2 (13 mL min^{-1}); H_2 flow rate, 33 mL min^{-1}; air flow rate, 360 mL min^{-1}; column temperature, 70^oC; detector temperature, 200^oC. A set of ethylene standards were also run, and C_2H_4 concentrations were determined by comparison. Ethylene detection was based on the retention time and quantification was done by comparison with the peak height from the standard ethylene concentrations.

Relative Growth rate

Wheat growth was measured in the form of the relative growth rate (RGR) between 70 and 90 days after seeding. Leaf area was measured with a leaf area meter (LI-COR, Inc., Lincoln, NE). Shoot dry biomass were recorded at 70 and 90 DAS, after oven drying at 65±2ºC in an oven for 72 hours. The RGR was calculated according to the formula used by (Hunt, 1982)

$RGR = [\ln (W_2) - \ln (W_1)] / (t_2-t_1)$

where W_1 and W_2 denote plant dry biomass at times t_1 and t_2.

Physiological parameters

Net photosynthesis (Pn), stomatal conductance (C) and intercellular CO_2 concentration were measured on the flag leaf using a portable photosynthesis system (CI-340 USA). The measurements were made on sunny days between 11 AM and 12 PM. The average photo synthetically active radiation (PAR), relative humidity and air temperature were: 800 µmol m^{-2} s^{-1}, 53% and 22ºC, respectively. Leaves used for photosynthetic measurement were divided into two halves for determination of nitrate reductase (NR) activity and ethylene production.

Nitrate reductase activity

The nitrate reductase activity in leaf was done according to the procedure of Hageman and Hucklesby (1971) with slight modifications. The fresh wheat leaf was cut into 2 mm slices and placed in ice-cold incubation medium containing 3 mL of 0.05M potassium phosphate buffer (pH-7.8) and 3.0 mL of 0.4M KNO_3 solution. The tubes were evacuated using a vacuum pump and then incubated in a 35 ºC water bath under dark conditions for 75 min. After incubation, tubes were kept in a boiling water bath for 5 min to cease the enzyme activity and complete the leaching of the nitrite into the medium. Nitrite was estimated by the method of Evans and Nason (1953). The aliquot (0.2 mL) from the reaction mixture was taken and 1 mL of 1% sulphanilamide in 1N-HCl and 1 mL of 0.025% N-1-Napthyl-ethylene diammonium dichloride in distilled water were added. The color was allowed to develop for 30 min after which the volume was made up to 6 mL with distilled water. The absorbance was read at 540 nm, using a UV-VIS spectrophotometer (Jenway 6300). The calibration curve was prepared using a sodium nitrite solution. The enzyme activity was expressed as µ mol NO_2 g^{-1}fw hr^{-1}.

Leaf N concentration

Leaf N concentration was determined after acid digestion of plant material by the Kjeldhal method (Bremner and Mulvaney, 1982).

Data analysis

Statistical analysis of the data was done by Duncan's Multiple Range Test at the 5% level of significance (Steel and Torrie, 1980)

RESU LTS

Ethylene production

The ethylene production from plants grown under low N concentration was greater than that of plants grown with higher N. The maximum (26 µL cm^{-2} leaf h^{-1}) was recorded in the treatment having low level of

N and highest level of L-MET and the minimum (5.5 µL cm^{-2} leaf h^{-1}) was from the treatment with zero L-MET along with higher level of N added. This increase was 122% greater than control and 118% greater than the highest level of N. Treatments with added L-MET had enhanced ethylene production at different N concentrations (Fig. 1).

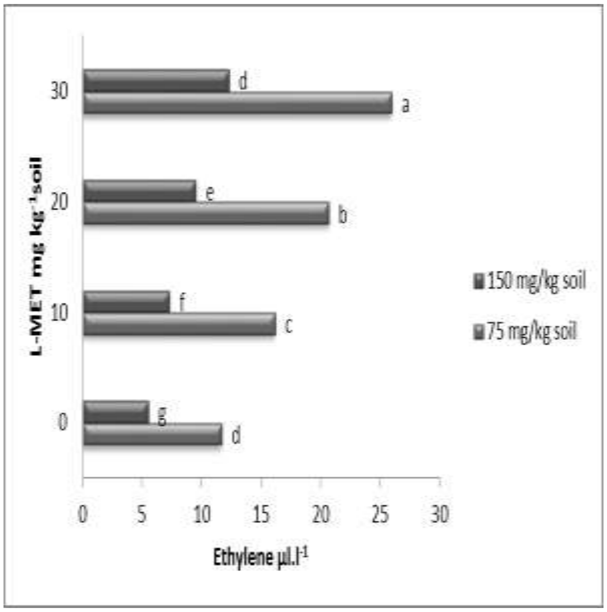

Fig. 1. Effect of foliar application of L-methionine on ethylene production by wheat leaves at two nitrogen Levels.

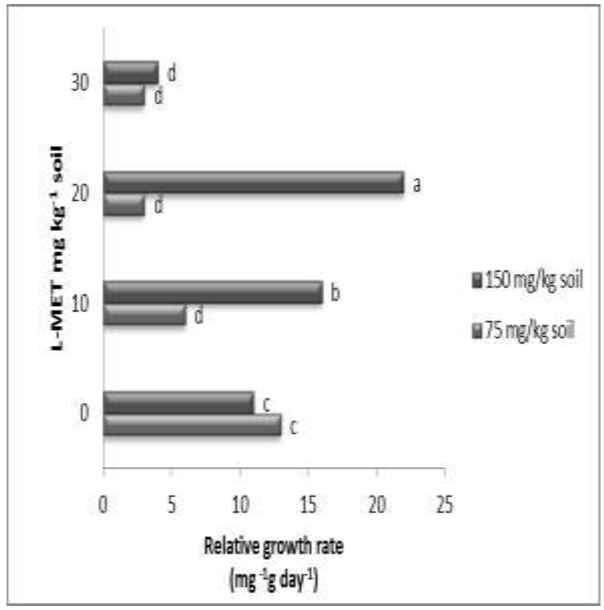

Fig. 2. Effect of foliar application of L-methionine on relative growth rate of wheat at two nitrogen levels.

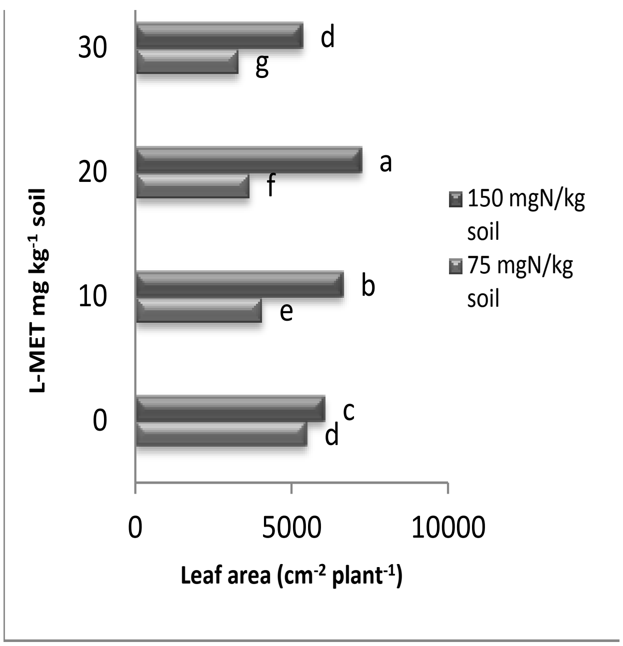

Fig. 3. Effect of foliar application of L-methionine on leaf area of wheat leaves at two nitrogen levels.

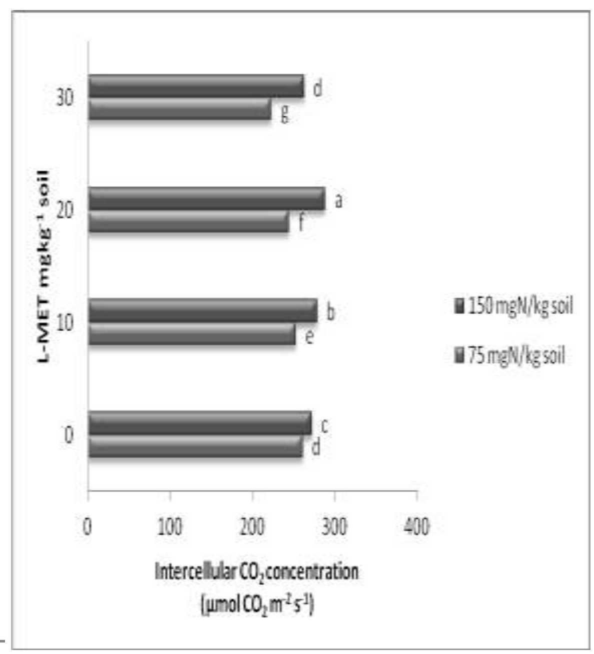

Fig. 4. Effect of foliar application of L-methionine on internal CO_2 concentration of wheat leaves at two nitrogen levels.

Relative Growth Rate & Leaf area

The maximum increase in leaf area and RGR was observed when L-MET was applied at 20 mg kg^{-1} soil, with the higher level of N. The minimum increase in leaf area was observed in the treatment with highest level of L-MET. Overall, the maximum increase (100%) in RGR over control was also in the treatment with 20 mg of L-MET and the higher concentration of N. It was also observed that the highest concentration of L-MET decreased leaf area at both levels of N. The overall increases in leaf area at the higher level of N relative to the lower levels were 10.4%, 64.7%, 99.7% and 63.7%, respectively (Fig. 2, 3).

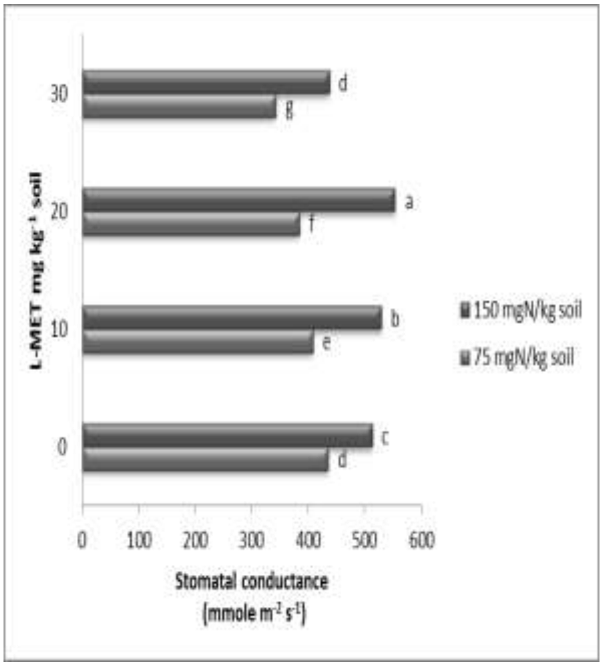

Fig. 5. Effect of foliar application of L-methionine on stomatal conductance of wheat leaves at two nitrogen levels.

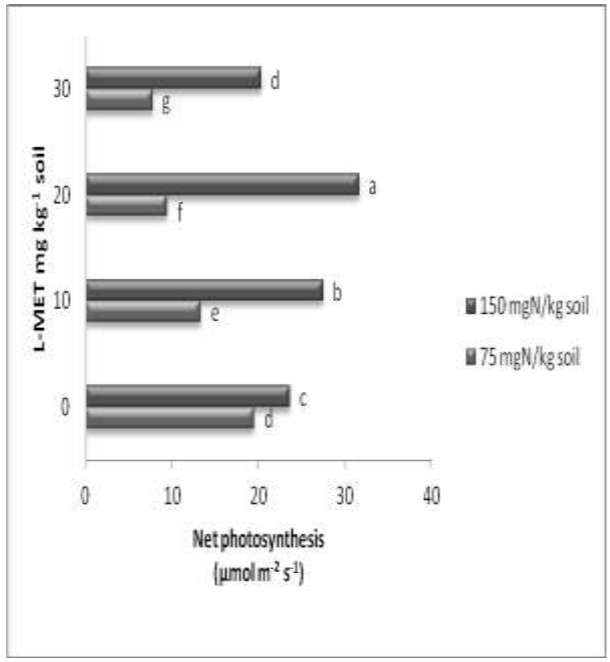

Fig. 6. Effect of foliar application of L-methionine on net photosynthesis of wheat leaves at two nitrogen levels.

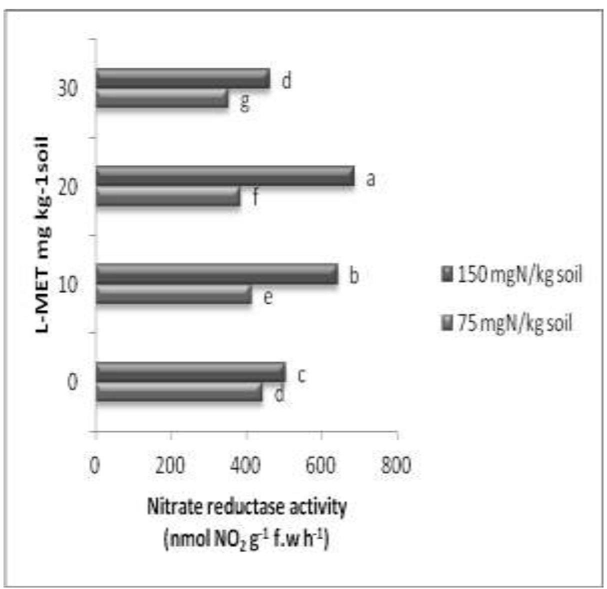

Fig. 7. Effect of foliar application of L-methionine on nitrate reducatse of wheat leaves at two nitrogen levels

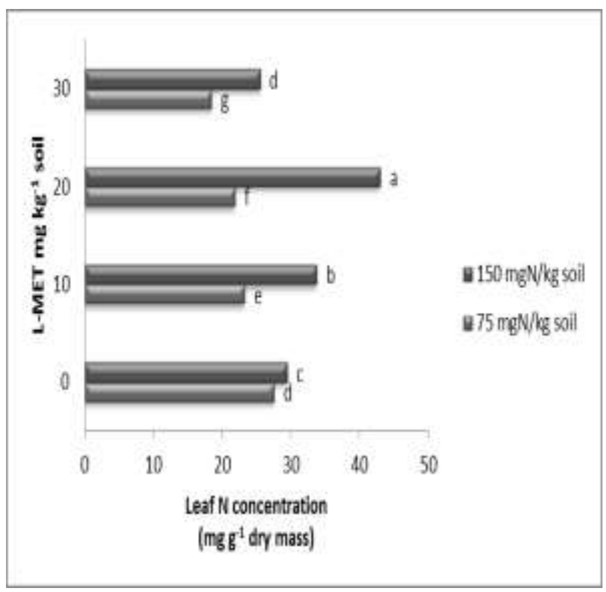

Fig. 8. Effect of foliar application of L-methionine on leaf nitrogen concentartion of wheat at two nitrogen levels.

Physiological parameters

Compared with the control, treatment with 20 mg L-MET kg^{-1} soil showed a strong stimulating effect on physiological parameters when applied with the higher N concentration, while a decrease was recorded at highest concentration of L-MET. However, at lower N levels, net photosynthesis decreased with increasing concentrations of L-MET, as compared to the control (Fig.4, 5, 6). Overall increases in these parameters at the higher N level were 21%, 10.7%, 23.6% and 16% greater when compared to the lower level. The same trend prevailed in the case of stomatal conductance and intercellular CO_2 concentration for plants grown with higher N levels.

Tissue N concentration and NR activity

The L-MET application at 20 mg kg^{-1} soil in the presence of higher N level increased tissue N concentration and NR activity (Fig. 7 & 8). The maximum tissue N concentration (38.9 mg g^{-1} dry mass) and NR activity (687.7 nmol NO_2 g^{-1} f.w h^{-1}) were 46% and 36% greater than the control. There was overall decrease of tissue N and NR activity that accompanied the application of L-MET at higher concentration (30 mg kg^{-1} soil).

DISCUSSION

The objective of this study was to evaluate the effect of nitrogen availability on the production of ethylene in wheat and to study how ethylene influences physiological parameters, growth and nitrogen accumulation in leaf tissue. It was noted that at the lower level of nitrogen, there was more production of ethylene than at the higher nitrogen level, and the treatment with L-MET (10-30 mg kg^{-1} soil) further enhanced ethylene production. The maximum increase in photosynthesis and growth were observed with higher level of nitrogen when applied with 30 mg of L-MET kg^{-1} soil. It can be inferred that L-MET treatment influences ethylene production depending upon the amount of soil available nitrogen, and influences the growth and photosynthetic response of wheat plants. As ethylene is a growth regulator, it enhances growth, yield, and cell expansion at lower concentrations (Khan, 2005). However, the findings of this study are in contrast with those of Tholen *et al.* (2004) who reported that ethylene-insensitive genotypes of *Nicotiana tabacum*, *Petunia x hybrid* and *Arabidopsis* did not show increase in total leaf area over a normal ethylene sensitive control.

During leaf expansion stages, the role of ethylene has been studied physiologically by using ethylene inhibitors and genetically by using transgenic plants or ethylene insensitive mutants. The results showed that the enzymes playing key role in the biosynthesis of ethylene did not express in these studies (Oh *et al.* 1999). In this study the ethylene imparted a promoting effect on RGR. Initially, RGR was similar to the control: having no ethylene treated with two levels of nitrogen. RGR doubled with the application of 20 mg of L-MET kg^{-1} soil, at the higher level of nitrogen. However, the higher level of L-MET reduced all parameters under study at both levels of nitrogen. These findings are not in line with those of Tholen *et al.* (2004) who reported no increase in RGR for ethylene insensitive plants. The studies on *Brassica juncea* by Lone *et al.* (2010) and Mir *et al.* (2010 a & b) reported that ethrel did not produce any significant effect at lower rates of nitrogen, but higher rates of ethrel affected growth parameters like leaf area, leaf area index, and total dry matter, thus resulting in more solar radiation being retained, which enhanced net photosynthetic rate.

The increase in photosynthesis by the application of L-MET at 30 mg kg^{-1} soil may be because of increased stomatal conductance and higher photosynthates accumulation (Ahmad *et al.* 2012). Many other studies have reported that ethylene influences the rate of photosynthesis because of variable rate of stomatal conductance (Khan, 2004; Khan *et al.* 2007). Significant differences in photosynthesis coupled with small changes in intercellular CO_2 concentrations were observed. It can be inferred that photosynthesis is dependent on other factors besides stomatal conductance. Studies conducted by Khan *et al.* (2007) showed that lower amount of nitrogen was transferred to Rubisco in ethylene-insensitive tobacco plants. This differentiated the lower capacity of ethylene-insensitive plants to their wild type. Based on the above statement, it can be suggested that ethylene at lower concentrations stimulates photosynthesis by moving photosynthates to the photosynthesis machinery (Khan *et al.* 2007). Lawlore (2001) and Marshner (1995) have described this aspect of nitrogen on photosynthetic enzymes and this cannot only be ascribed to conversion of L-MET to ethylene because plants produce ethylene endogenously and its conversion to ethylene glycol and ethylene oxide has already been taking place in plants at specific locations (Arshad and Frankenberger Jr., 2002).

Leaf nitrogen concentration and leaf NR activity were increased by L-MET when applied at 20 mg kg^{-1} soil along with higher nitrogen level. There are several cases of nitrogen and ethylene interaction as reported by Baker and Corey (1990), which sand or soil culture nitrogen equally produced ethylene and increased shoot dry biomass in tomato or as reported by Ahmad et al. (2009a) that CaC_2 improves nitrogen availability by inhibiting nitrification/de-nitrification and improves growth and yield in wheat. In many cases nitrogen availability affects ethylene production and responsiveness (Lynch and Brown, 1997). Tari and Szen (1995) reported that under nitrogen deficient conditions ethylene evolution increased in wheat seedlings.

Conclusion

The present study indicated that L-MET is an effective source of ethylene, and that ethylene production is affected by nitrogen availability and it has a positive influence on photosynthesis, leaf nitrogen concentration and growth of winter wheat. Plants grown with lower levels of soil nitrogen produce more ethylene than the plants having more available nitrogen. As L-MET concentration increases, there is a response in an increase of ethylene production. In the higher N application rate, the increase in L-MET was also generally tied to an increase in relative growth rate. While CO_2 concentration did not appear to be strongly related with L-MET application rate, there were small changes between different treatments, though no pattern was readily available. However, photosynthesis rates exhibited large changes in response to changes in L-MET concentration. It is important to note that, with the exception of ethylene production, all parameters measured for the lower rate of N application (75 mg N kg^{-1} soil) exhibited a decrease. In contrast, for the higher rate of N application (150 mg N kg^{-1} soil), all parameters except ethylene showed an increase up to 20 mg L-MET kg^{-1} soil, and then a decrease at 30 mg L-MET kg^{-1} soil.

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