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# Changes in Soil Fertility Indicators of Paddy Soil in Response to Metsulfuron-methyl Application

**Laliteshwari Bhardwaj***

*Department of Botany, Institute of Science, Banaras Hindu University, Varanasi-221005, India*
**Correspondence E-mail: laliteshwaribhardwaj@gmail.com*

***Abstract:*** Metsulfuron methyl a selective herbicide, chemically refereed as 2-[(4-methoxy-6-methyl-1,3,5-triazin-2-yl) carbamoylsulfamoyl], widely used in cereals, fruits and vegetables to control weeds. Its continuous application is expected to influence soil fertility by alteration in various biogeochemical processes. Present research was conducted to investigate the effect of Metsulfuron-methyl (MSM), applied at three different doses (LD: lower dose; RD: recommended dose and HD: higher dose) on soil fertility indicator parameters in a rice agro-ecosystem. MBC (µg $g^{-1}$soil) varied from 198 to 221 (LD treated soils); 189 to 192 (RD treated soils) and 171 to 179 (HD treated soil) compared to control soils (225 to 230). Study reveals that MSM influenced greater at higher dose treated soils followed by LD and RD treated soils. Outcomes derived through this study have immense significance in formulations of strategies for management of rice agro-ecosystems for judicious application of dose dependent herbicides.

***Keywords:*** Metsulfuron-methyl, microbial biomass, soil respiration, soil fertility, paddy soil

## 1. INTRODUCTION

Rice (*Oryza sativa* L.), predominantly is the most important cereal crop across the globe. India is recognized as the second largest producer of rice after China (FAO, 2014). Weeds infestation is a major constraint to rice cultivation. As per the report, loss shared by weeds is 34% out of total pests whereas herbicides used is only 16% which is very less in comparison to other industrialized countries (Chaudhary et. Al., 2014). It is estimated that there could be up to 47% reduction in paddy crop yield without weed control management. (Oerke, 2005). Therefore, to overcome weed infestation, application of array of herbicides has become a common practice globally (Rose *et al.*, 2015). Although application of herbicides enhanced the rice yield but its continuous application of herbicides would be detrimental to soil fertility and leads to other environmental issues. Therefore, this issue has attracted attention of various researchers to address the problem of agro-ecosystem arising due to herbicides. Pertaining literature suggested that various efforts have been made related to this aspect targeted influence of herbicides on various soil attributes (Sofo *et al.*, 2012; Zain *et al.*, 2013). Therefore, assessment of effect of herbicides on soil fertility parameters is logical and imperative. A comprehensive study is required to determine the selected herbicide residues in soil so that a well-developed idea can be generated on the effect of their metabolites. Although many work related to the effect of sulfonylurea herbicides on soil microbiota, enzyme activity, respiration and microbial biomass have been done so far but less studies are reported for metsulfuron meythyl. (Sofo *et al.*, 2012; Zain *et al.*, 2013; Ljiljana *et al.*, 2014). There are various soil health indicators to evaluate the overall quality and fertility of soil (Anderson, 2003; Bloem *et al.*, 2005).

As per the report of Ismail *et al.* (1996) soil respiration and microbial biomass declined at higher doses whereas no significant effect was observed at field rate. Fate and metabolic

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degradation pathway of metsulfuron methyl have largely been studied till date (Pons and Barriuso, 1998; Ghani and Wardle, 2001; Sondhia, 2009). Singh and Paul (2011) have been reported 70% recovery of metsulfuron- methyl in soil. Wang *et al.* (2009) reported that metsulfuron-methyl has the persistence of many months in the environment. Sofo *et al.* (2012) found that all the selected sulfonylurea herbicides at 10-fold higher rate had stronger deleterious effect on, microbial biomass carbon, soil respiration and soil enzyme activities than applied at the field rate of herbicides. Lupwayi *et al.* (2003) determined microbial biomass carbon with respect to the application of metsulfuron-methyl. Loganayagi and Ramesh (2015) while determining soil nutrient content at 0.02 μL at 0.2 μL metsulfuron-methyl $kg^{-1}$ dry weight soil found the deviations between 4 and 18.8%, respectively, from control soil samples. Based on above literature reports it is clear that least attention has been made towards analyzing variations in soil fertility indicators in relation to MSM dose dependent application.

Therefore, present investigation made in a rice agro-ecosystems treated with metsulfuron-methyl with the objectives a) to measure the herbicide residue content, b) to determine the important soil fertility indicators, in response to three different doses of metsulfuron-methyl.

## 2. MATERIALS AND METHODS

### 2.1. Study site, experimental layout and soil sample collection

The proposed experiment was conducted at the Agricultural Farm, Banaras Hindu University, Varanasi, U.P., U.P., having latitude at 25°18′N and longitude at 83°31′E with 75.7 meters above the mean sea level altitude. Experimental plots ($5x3m^2$) were constructed in a randomized block design (RBD) pattern with three replicates of each treated and untreated plots. Field was planted with rice (*Oriza sativa*) cultivar Surju-52. A commonly used herbicide Metsulfuron-methyl was applied @ three different doses: 5, 20 and 60 mg $kg^{-1}$ soil as post-emergence chemical. Controlled soil left untreated. Soil sampling in triplicate was randomly done from 0-15 cm depth, using soil corer and auger. Sampling was done at 25, 35, 45, 65 and 85 days after application of herbicides in two consecutive years during 2018-19.

### 2.2. Methodology

#### *2.2.1 pH*

Soil pH was measured by electrometric method. The mixture of soil and water was prepared in the ratio 1:2.5 and mixed well on shaker for one hour. Measuring of soil pH was done by pH meter armed with glass electrode (Cyber Scan pH 510, Eutec Instruments Pte Ltd).

#### *2.2.2. Total organic carbon*

The assessment of total organic carbon was done based on the principle of dichromate oxidation/ digestion in which soil was digested using tri-acid solution ($HClO_3$, $HNO_3$ and $H_2SO_4$). Ferrous ammonium sulphate was used as titrant for digested soil as per procedure of Walkley (1947).

#### *2.2.3 Soil moisture*

Gravimetric method was adapted to determined soil moisture content. Soil samples dried at 105° C on oven until achieving constant weight.

#### *2.2.4. Mineral-N*

Mineral-N was determined by combining the values of ammonium-N and nitrate-N in soil. Exchangeable ammonium ($NH_4^+$-N) was determined using 2M KCl extracts and analyzed spectrophotometrically by phenate method (APHA, 1985). Estimation of Nitrate ($NO_3$-N) was done by phenoldisulphonic acid and estimated spectrophotometrically at 420 nm.

#### *2.2.5 Microbial biomass analyses*

Microbial Biomass Carbon (MBC) and Nitrogen (MBN) were measured using chloroform-fumigation-extraction methodology. Detailed protocol mentioned elsewhere (Brookes *et al.*, 1985; Vance *et al.*, 1987). Pre-conditioning of fresh soil samples (sieved) was done in air tight jar at room temperature for a week. Soil fumigation with chloroform (ethanol free) was done for two days and extracted using 0.5 M $K_2SO_4$. Soil MBC was calculated according to formula MBC= 2.64

EC in which EC : Extracted organic C differences between fumigated and non-fumigated soil samples which is expressed in terms of $\mu g\ g^{-1}$ dry soil (Vance *et al.*, 1987). For MBN estimation the equation MBN= EN/0.54 was employed.

### 2.2.6. Metsulfuron-methyl residue analysis

Extraction of was done as described by Sondhia (2008). After filteration, the extracts were collected and evaporated on rotary evaporator. The residue was reconstituted in acetonitrile and thereafter analyzed by HPLC. The HPLC was fitted with 100A (50 × 4.6 mm) C18 column. The detection of metsulfuron-methyl was done at 220 nm. The mobile phase used was Acetonitrile: water (70: 30). The flow rate during elutions was maintained at 1 mL $min^{-1}$. Retention time of metsulfuron-methyl was found approximately 2.54 min.

### 2.2.7. Bacterial and fungal population count

Soil dilution plate technique was opted for estimating total culturable bacteria and fungi using tryptic soy agar for determining bacterial population and Czapek agar for fungal community. The inoculated agar Plates were taken in triplicate for each soil samples and were incubated at $28^0$C for 5 days for bacteria and a couple of weeks for fungal population, and then the colonies were counted (Archives for Technical Sciences, 2014).

### 2.2.8. Statistical analyses

Effect of treatments on targeted parameters was analyzed using ANOVA. Level of significance was least $p<0.05$ in all the analyses. Relationship among soil variables were established using Linear regression analysis. Tukey's HSD test at 5% level of significance was used for separating the treatment means. SPSS 22.0 (SPSS Inc., 2013) was used for statistical analysis.

## 3. RESULTS

### 3.1. Soil physicochemical properties

Soil properties like texture, pH, water holding capacity (WHC), total nitrogen and carbon were estimated once initially before analyzing other seasonal parameters. The soil type is Inceptisol with sand 32%, silt 65% and clay 3% with soil texture Sandy clay loam. pH recorded was 7.05. WHC 43 %, Total nitrogen was found to be 0.13% and organic C was 0.68 %.

### 3.2. Variations in Soil moisture, Mineral-N and Microbial biomass carbon

Higher doses of MSM have the greater effect on soil moisture, MBC and MBN as shown in Table 1. The values of MBC declined significantly after the treatment with different doses of MSM are as follows: control> LD >RD >HD. MBC value in control reduced to ~16 % at RD of MSM whereas ~ 22% reduction was observed at HD. MBN had shown the similar pattern as MBC as LD and RD MSM had less effect on MBN compared to HD MSM. MSM at higher dose reduced up to 57% MBN of control value whereas 25% was curtailed at LD of herbicide and in case of RD it was reduced to 39.5% to the control value. Pattern of variations in recorded values for the consecutive years of the study was more or less similar to MSM treatments.

### 3.3. Bacterial and fungal population count

Changes in the total bacterial population with respect to herbicide application have been given in Table 2. Highest bacterial population (2.46 cfu/g soil $X10^8$) was recorded 45 DAA for control untreated soils as compared to treated soils (LD: $1.97x10^8$); (RD: $1.52x10^8$) and (HD: $1.14\ x10^8$). The fungal population size in the different dose treated soils showed in Table 3. Effect of LD of MSM on fungal population size was found insignificant whereas the maximum increase ($68.3x\ 10^7$) was observed at RD on 45 DAA. ANOVA revealed significant differences due to treatment in case of bacteria and fungi population.

### 3.4. Residue concentration of metsulfuron-methyl in soil

Table 4 exhibits the results of MSM residues applied at various rate in soil. Concentration of MSM residues declined gradually with due course of time period. Residues after 50 days were quantified 0.002, 0.011 and 0.010 $\mu g\ g^{-1}$ soil at LD, RD and HD, respectively. At 85 DAA the residue concentration was <0.001 $\mu g\ g^{-1}$ (below detectable level).

**Table 1: Values (mean ± SD) of soil moisture (%), mineral-N (µg g$^{-1}$), microbial biomass C (MBC: mg C kg$^{-1}$ soil), and N (MBN: mg N kg−1 soil), in paddy field soil**

| *Parameters* | *Soil moisture* | | *Mineral-N** | | *MBC** | | *MBN** | |
|---|---|---|---|---|---|---|---|---|
| *Year* <br> *T* | *2018* | *2019* | *2018* | *2019* | *2018* | *2019* | *2018* | *2019* |
| **Control** | 8.9±0.84 | 8.2±0.80 | 8.8±0.85 | 8.7±0.84 | 225±11.52 | 230±11.25 | 89±7.98 | 91±8.85 |
| LD* | 7.3±0.76 | 7.6±0.77 | 7.9±0.78 | 8.2±0.79 | 221±10.25 | 198±10.12 | 68±6.98 | 65±6.56 |
| RD* | 7.5±0.77 | 7.8±0.78 | 6.5±0.78 | 6.2±0.75 | 189±9.78 | 192±9.12 | 55±5.87 | 51±5.21 |
| HD* | 3.2±0.05 | 4.1±0.06 | 3.8±0.05 | 4.6±0.07 | 171±8.98 | 179±8.99 | 39±4.78 | 38±4.78 |

SD: standard deviation; * means difered signifcantly ($p < 0.05$).
*LD: lower dose; RD: Recommended dose; HD: higher dose of herbicide

**Table 2: Bacterial cfu/g soil (X10$^{8}$) in soil, treated with LD, RD and HD of MSM**

| *Metsulfuron-methyl (mg a.i.kg-1)* | *Days after application* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *20 2018 -19* | | *35 2018 -19* | | *45 2018 -19* | | *60 2018 -19* | |
| **Control** | 1.98±0.07 | 1.85±0.10 | 1.96±0.06 | 1.95±0.15 | 2.46±0.12 | 2.38±0.08 | 2.37±0.07 | 2.41±0.06 |
| **LD MSM** | 1.89±0.07 | 1.78±0.10 | 1.91±0.06 | 1.82±0.15 | 2.03±0.12 | 2.28±0.08 | 2.11±0.07 | 2.01±0.06 |
| **RD MSM** | 1.34±0.04 | 1.15±0.06 | 1.51±0.05 | 1.48±0.03 | 1.74±0.15 | 1.81±0.17 | 1.61±0.07 | 1.55±0.08 |
| **HD MSM** | 0.76±0.05 | 0.67±0.06 | 1.02±0.04 | 1.35±0.01 | 1.13±0.03 | 1.26±0.04 | 1.42±0.06 | 1.56±0.07 |

**Table 3: Fungal cfu/g soil (X10$^{6}$) in soil, treated with LD, RD and HD of MSM**

| *Metsulfuron-methyl (mg a.i.kg-1)* | *Days after application* | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | *20 2018 -19* | | *35 2018 -19* | | *45 2018 -19* | | *60 2018 -19* | |
| Control | 3.53±0.05 | 3.48±0.15 | 3.23±0.06 | 3.31±0.12 | 3.56±0.07 | 3.48±0.01 | 3.85±0.12 | 3.79±0.06 |
| LD MSM | 3.52±0.08 | 3.5.4±0.13 | 28.1±0.04 | 2.69±0.02 | 3.92±0.16 | 3.75±0.11 | 4.65±0.08 | 4.35±0.04 |
| RD MSM | 5.58±0.17 | 5.45±0.17 | 5.58±0.17 | 5.47±0.17 | 6.53±0.17 | 6.83±0.17 | 6.21±0.17 | 6.23±0.17 |
| HD MSM | 6.51±0.17 | 6.78±0.17 | 6.38±0.11 | 6.82±0.17 | 5.93±0.17 | 5.73±0.17 | 4.57±0.17 | 4.48±0.17 |

**Table 4: Detection of Metsulfuron-methyl residues in paddy soil**

| *Treatment MSM (mg a.i.kg-1)* | *Days after application* | | | | | |
|---|---|---|---|---|---|---|
| | *Residues (µg g$^{-1}$)* | | | | | |
| | *25 2018 - 2019* | | *50 2018 -19* | | *85 2018 -19* | |
| Low dose | 0.009±0.002 | 0.008±0.001 | 0.002±0.001 | 0.001±0.001 | *BDL | BDL |
| Recommended dose | 0.013±0.007 | 0.016±0.010 | 0.011±0.005 | 0.012±0.004 | BDL | BDL |
| High dose | 0.019±0.012 | 0.020±0.011 | 0.010±0.008 | 0.008±0.001 | BDL | BDL |

* BDL: Below detection limit

## 3.5. Soil respiration

Soil respiration data for 85 days incubation period, during the year 2018 and 2019, in response to studied herbicide, are show in Fig. 1A and 1B, respectively. At recommended dose of MSM soil respiration value was found maximum (80 mg C-$CO_2$ kg$^{-1}$ soil) as compared to LD (67.2), HD (68.8) and control (76 mg C-$CO_2$ kg$^{-1}$ soil). Similar trend was observed across the years.

## 4. DISCUSSION

Understanding towards interaction of soil fertility parameters with herbicides is utmost important and logical to make the sustainable strategies for judicious use of chemicals in the agro-ecosystem. Soil pH is an underling soil variable that influences many physical, chemical and biological properties of soil. In present study metsulfuron-methyl was found below detectable

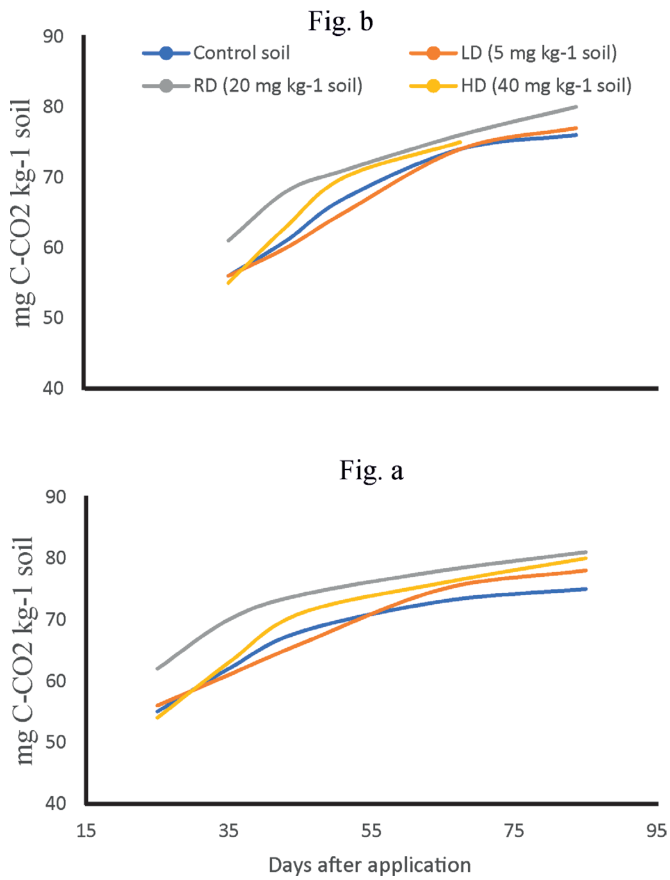


**Figure 1. Cumulative values of soil respiration at LD, RD and HD of MSM at varied incubation period during a. 2018 and b. 2019**

level after 50 and 85 days across all doses of MSM as the soil samples were slightly alkaline (pH 7.05). With the increase in pH, adsorption of sulfonylureas decreases Mehdizadeh M, *et al.* (2017)). Soil pH plays crucial role in degradation of sulfonylurea herbicides (Morrica *et al.* 2001). Timothy *et al.* (2012) reported that soil with slightly acidic nature was capable of absorbing more sulfonylurea herbicides.

Several studies reported that ~ 48% of MSM are transformed into bound residues in the form of primary and secondary metabolites in soil (Pons and Barriuso 1998; Ye *et al.*, 2003). Sondhia (2008) observed that with due course of time, gradual reduction of herbicide concentration in soil was due to microbial activities and other processes. Microbial population residing in soil is mainly responsible for degrading sulfonylurea by catalyzing the pathways associated to herbicides degradation (Molinari *et al.* 1999). These observations are corroborated to present finding where rate of dissipation of MSM was faster enough to not get detected with due course of time duration after 50 days. Minimum MBC value was recorded in soil with maximal concentration of MSM residues. Metsulfuron-methyl treated soil at all doses depicted reduced MBC and MBN in comparison to untreated soil. Perucci *et al.* (2000) have also found similar trends. Reduction in MBC (~25-65%) have also been determined by Vischetti *et al.* (2002) following imazamox and benfluralin herbicides application. Abbas et. al. (2014) has suggested that decrease in MBC occurs probably due to soil organic matter reduction. Changes in MBN content showed similar pattern as that in case of MBC. Greater residual content of herbicide at higher dose leads to decline in soil microorganisms which further declines MBN values. Another possibility of MBN reduction is due to higher susceptibility of $N_2$- fixing bacteria to herbicide mainly at higher doses (Abbas *et al.*, 2014). Such types of finding have also been endorsed by Nada *et al.* (2002) based on experiments with *Azotobacter* sp.

The present study revealed that bacterial population varied with the trend as highest in untreated soil, followed by lower, recommended and higher dose of herbicide treated soils (Table 2). Abbas *et al.* (2014) investigated that application of post-emergence herbicide leads to reduction in bacterial population size. The reports of Sebiomo *et al.* (2011) also indicated that due to soil amendment with selected herbicides total bacterial population diminished. Heterotrophic bacterial population got reduced in number due to application of metsulfuron-methyl (He *et al.*, 2006). Drop in the bacterial abundance also be due to suppressed rhizobial growth and proliferation in response to herbicide (Abbas *et al.*, 2014).

The fungal population in this study obtained least in control soil, followed by low dose, recommended and higher doses metsulfuron-methyl treated soils. The fungal populations were significantly ($p<0.05$) increased in the presence 25 and 50 mg of metsulfuron-methyl (Table 3).

Ljiljana *et al.* (2014) also reported significant increase in fungal abundance at RD and at higher dose than low dose and untreated soil. The same findings were depicted by others (Ahtiainen *et al.*, 2003; Zabalou *et al.*, 2004).

Numerous studies (Startton *et al*., 2003; Ratcliff *et al*., 2006) related to soil microbiota revealed the role of soil physicochemical characteristics in altering the number and biological activities of microorganisms. The present study disclosed that herbicide exerted positive role in enhancing soil respiration in comparison to control (Fig. 1a and 1b). Soil respiration could not reveal a clear-cut pattern towards the applied herbicide as higher dose could produce more CO2 than other doses applied in the field. Pertile *et al.* (2020) observed that soil respiration was not significantly changed in response to herbicides, Flumioxazin and imazethapyr at varying incubation period.

## 5. CONCLUSION

It can be inferred that metsulfuron-methyl is safe to apply at low and recommended dose because its effect on soil fertility parameters were found least affected. Outcome derived through present communication will be useful in formulating strategies for safe application of herbicides with special reference to quantity/dose dependency.

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