Research Article

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Assessment of methyl 2-({[(4,6-dimethoxypyrimidin-2-yl)carbamoyl] sulfamoyl}methyl)benzoate through biotic and abiotic degradation modes

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Abstract: Detoxification and management of environmental contaminants is an exigent issue of current times. Sulfonylurea herbicide, Bensulfuron-methyl was investigated for its degradation demeanour in soils, through biotic and abiotic modes (biodegradation and hydrolysis). Solid-liquid extraction of the herbicide was followed by GC-MS and UV-visible spectrophotometry analysis. The main metabolites observed were pyrimidinamine [149 m/z] and benzylsulfonamide [182 m/z]. The rate of biodegradation achieved by *Aspergillus niger* and *Penicillium chrysogenum* was 95% and 71%, respectively. The maximal decline in Bensulfuron-methyl concentration through hydrolysis was 48%. Furthermore, hydrolytic elimination was also evaluated based on time and pH. Both these parameters had a strong influence on the rate of transformation. Soils with lower pH exhibited an increased rate of degradation while a temperature of 27±2°C gave ideal conditions for herbicide decomposition. Percentage degradation and rate constant (k) followed first order reaction kinetics. Non-inoculated soils displayed less amounts of degradation. Furthermore, relative standard deviations were calculated for the residuals extracted in all soils. Analysis of variance (ANOVA) provided a p value < 0.05 for both strains with R^2 closer to 1 signifying the significance of the results. Both fungal strains proved their potential for Bensulfuron-methyl remediation in soils.

Keywords: sulfonylurea; GC-MS; metabolites; hydrolysis; herbicide.

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1 Introduction

The cardinal function of pesticides is the elimination of pests, not only in the rural agricultural setups but in urban dwellings as well [1]. Their extensive application is being met with some serious implications regarding their persistence in environment as well as their toxicological effects on non-targeted species [2]. The boundless use of pesticides is raising formidable issues, as only < 5 % of these agrochemicals applied, actually reach their desired destination [3]. The remains of the applied pesticide are more commonly present in surface and ground waters. Due to their mobile nature, these pesticides contaminate diverse layers of soil and environment through leaching, volatilization or sorption. Consequentially, they can reach water bodies either directly by runoff from agricultural fields or indirectly through industrial wastes [4]. Pesticides, which promptly degrade naturally in environment, are non-persistent while those that do not degrade easily are persistent. The persistent agrochemicals are responsible for the detrimental effects on the environment due to their prolonged stay. They are also responsible for creating resistance in pests towards them [5]. The degradation of pesticides involves different routes, which results in the decontamination of the environmental system at varying rates. Expediting the rate of degradation causes faster detoxification of the environment in lesser time. Decontamination of soils and water bodies from these pesticides is thus crucial [6]. However, physicochemical modes of decontamination such as land filling, incineration or recycling are less productive and costly [7]. Hence, photo-degradation, biodegradation and hydrolysis are more dominant methods for pesticides removal. Among these, biodegradation is paramount for removal of toxic chemicals, as microbes are present in abundance in nature [8]. The survival of microorganisms is mostly dependent on the bioremediation phenomena,

as they consume these chemicals as a source of nutrients. Soils and water bodies can be decontaminated either by naturally occurring microbes in the environment or by bio-augmentation of these environments with microbes [9]. This process is highly beneficial due to less damage to the ecosystem whilst being more economical and is used in several countries globally. Microbes are among the main degrading microbes of xenobiotics including pesticides. In the biodegradation phenomena, several enzymes and gene clusters are involved [10]. In recent advances, bacteria with engineered genes are used in the degradation of pesticides. Several fungal strains including, *Aspergillus niger*, *Fusarium verticillioides*, *Fusarium oxysporum*, *Lentinula edodes*, *Penicillium brevicompactum* and *Lecanicillium saksenae*, have been isolated and screened, which have been further utilized for removal of toxic substances including endosulfan, terbuthylazine, difenoconazole, and pendimethalin and several sulfonylurea herbicides. To boost up the degradation process a mixture of fungal strains in the form of a consortium is also utilized [11].

Sulfonylurea herbicides display a positive response towards microbial degradation. One such example is the degradation of nicosulfuron by *Pseudomonas flourescens*, isolated from agricultural soils. 77.5% Nicosulfuron was degraded by the bacterial strain [12]. Berger and Wolfe were successful in degrading 12 sulfonylurea herbicides. According to their results, microbial degradation was dominant at neutral pH while hydrolysis occurred at low pH values [13]. Furthermore, Pergal et al. also studied nicrosulfuron and thiensulfuron methyl degradation recently through chlorine dioxide in water [14]. An impressive degradation was achieved for metsulfuron methyl by enzymatic action. A degradation rate of 93% was achieved after just 10 min [15]. A mixture of sulfonyurea herbicides was degraded by *B. megaterium*, isolated from an agricultural soil [16].

Bensulfuron-methyl (BSM), a sulfonylurea herbicide with chemical name, Methyl α-(4,6-dimethoxypyrimidin-2-ylcarbamoylsulfamoyl)-o-toluate, is a white to light yellow, fine crystalline powder. Although applied in low quantities, BSM has high application rates [17]. It targets on weeds like *Cyperaceae, Butomaceae* and *Alismataceae* growing in the rice fields [18,19]. A weed specie *Sagittariatrifolia L* has been found to be resistant to BSM due to mutation in acetolactate synthase gene [18]. It was found to be accumulated in brown rice and rice straw because of its persistent nature [20]. At neutral pH, the half-life of BSM is approximately 143 days [21]. It has been found to be degraded by several microbes including *Ochrabacterium sp.*, *Brevibacterium*, *Bacillus megaterium*, *Methylopila sp.* among others [22,23].

A massive use of pesticides is observed in the agricultural country of Pakistan especially herbicide Bensulfuron-methyl, because of the abundance of crops grown such as wheat and rice. In order to understand BSM mitigation in contaminated soils, it is necessary to study its degradation behaviour in environment. The current research investigates the degradation trend of BSM including bioremediation and hydrolysis in soils with distinct physicochemical properties utilizing two fungi: *Aspergillus niger* and *Penicillium chrysogenum*. Metabolites obtained from BSM degradation can be utilized in prediction of the toxicological fate of this herbicide in soil.

2 Experimental

2.1 Reagents

Bensulfuron-methyl (ACCU, USA), anhydrous Na_2SO_4 (Sigma-Aldrich, Germany) and dichloromethane (DCM) (RCI-Labscan, Thailand).

2.2 Fungal strains

Two fungal strains; *Aspergillus niger* and *Penicillium chrysogenum*, were contributed by the Mycotoxicology Lab, Environmental Sciences Department, Fatima Jinnah Women University, Rawalpindi, Pakistan. Fungi were cultured in petri plates comprising PDA inside a laminar flow hood and incubated at 29°C for 5 days.

2.3 Soil samples

Biodegradation experiments were conducted on four soils from various locales of Pakistan encompassing, Bahawalpur (Soil 1), Swabi (Soil 2), Mianwali (Soil 3) and Pasni (Soil 4) from a depth of 0-20 cm. The physical and chemical analysis of soil samples was done by Soil and water testing laboratory, Pakistan. Soil tests underwent further processing [24]. Results of biodegradation were analysed by gas chromatography mass spectrometry (GCMS-QP5050 (SCHIMADZU)) and UV-Vis spectrophotometer (BMS-1602, Biotechnology Medical Services K. Group, U.S.A). A pesticide stock solution of 10 ppm concentration was prepared for biodegradation experiments while a dilution of 1 ppm for hydrolysis experiments was prepared.

2.4 Soil Mycoremediation experiments

Approximately 5 g soil, 2 ml BSM initial solution and 10 ml spore suspension of both *A. niger* (6.3 x10^5 spores ml^{-1}) and *P. chrysogenum* (6.0 x10^5 spores ml^{-1}) were added in individual glass vials. Control samples were prepared devoid of fungal suspension with only containing pesticide and soil solution. Moisture content was maintained at 70-80% of WHC. Capped tubes were vortexed at 20(x100) rpm to ensure a homogenous mixture. The vials were kept in incubator (27±2°C) for 21 days. Sampling was done from each vial at multiple intervals (7, 14 and 21 days). Experimentation was performed in duplicates [25]. Fungal growth was observed and calculated by using a haemocytometer under a microscope on the days of extraction.

2.5 BSM hydrolysis

Hydrolysis experiments for BSM degradation in both soil and water were performed following the technique used by Sarmah et al. [26].

2.6 Pesticide extraction

Extraction was performed using 5 ml DCM twice, followed by evaporation. Followed by evaporation, 1.5 ml extractant was stored in eppendorf tube. These tubes were put in refrigerator at -4°C until further analysis [27].

2.7 Chromatographic investigation

The samples were examined by GC–MS (QP5050A, Shimadzu, Japan) system equipped with a DB-5MS fused quartz capillary column (30 mm × 0.25 mm × 0.25 µm). The temperature program was set as following: initial temperature 150°C held for 2 min up to 250°C at 10 °C/ min (maintained 10 min). Injector temperatures was maintained at 280°C. Helium was used as carrier gas at a flow rate of 1.2mL/ min.

2.8 UV-Visible spectrophotometer analysis

BSM hydrolysis rate was analysed by UV-Visible spectrophotometer (UV-BMS 1602). Full scan at wavelengths of 190-250 nm was run for each sample. Bensulfuron-methyl peak was attained at 200 λmax (nm).

2.9 Degradation percentage and kinetics

The rate of BSM degradation was determined after GC-MS analysis. Means of the duplicate values of samples were utilized. Percentage degradation of BSM was calculated by the following formula (Equation 1):

$$B_t = \frac{D_b - D_a}{D_b} \times 100 \quad (1)$$

where, B_t is the degradation of BSM in percentage at a precise time t, D_a is the residual pesticide left in the sample after time t and D_b is the quantity of pesticide in control sample [28]. The pseudo-first order rate constant for the degradation of BSM was determined by the integrated arrangement of the first order reaction kinetics (Equation 2).

$$lnC_t = -kt + lnC_\circ \quad (2)$$

where, C_t is the pesticide concentration at time t, $C_\circ$ is the pesticide concentration at the start and k is the degradation rate constant.

The rate constant, k was obtained by the following equation (Equation 3).

$$Rate(k) = -\frac{\Delta[C]}{\Delta t} \quad (3)$$

Half-life was determined by Equation 4,

$$t_{1/2} = \frac{0.693}{k} \quad (4)$$

The data was subjected to statistical analysis for further confirmation of results.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Soil properties

Physical and chemical properties of soil were studied and analysed from four regions of Pakistan. These locations displayed variable physical and chemical properties (Table 1). The effects of diverse soil characteristics on pesticide biodegradation and hydrolysis were assessed. Soils in the current experiment displayed a neutral to alkaline trend with only one soil exhibiting slight acidic pH. Soils of this region have been reported previously to be generally

Table 1: Physical and chemical characteristics of soil samples.

Soil	pH	OM[1] (%)	TN[1] (%)	TOC[1] (%)	WHC[1] (ml l^{-1})	CEC[1] (Meq100g^{-1})	EC[1] (µScm^{-1})	Pb (ppm)	Cd (ppm)	Ni (ppm)	Cu (ppm)	Zn (ppm)
1	7.4	2.7	0.13	1.5	66	7.8	240	3.13	0.68	0.77	0.17	0.20
2	7.3	2.3	0.36	1.3	60	7.5	120	0.72	0.06	0.68	N.D	0.09
3	6.6	3.3	0.31	1.1	48	7.8	84.0	3.31	1.16	0.73	N.D	0.08
4	8.6	1.8	0.09	1.0	52	7.0	232	3.47	0.15	0.43	0.04	0.08

[1]OM= Organic Matter, TN= Total Nitrogen, TOC= Total Organic Carbon, WHC= Water Holding Capacity, CEC= Cation Exchange Capacity, EC= Electrical Conductivity.

alkaline in nature. Basic pH of soil facilitates the accruing of a higher concentration of salt, which affects soil matrix causing capriciousness and has a detrimental impact on it. According to the physicochemical characterisation, soil 3 possessed the lowest pH (6.6) while the highest pH value was found in soil 4 (8.6). An opposite trend for organic matter (OM) was observed in these soils, with soil 3 showing maximum organic matter (3.3%) and soil 4 displaying the least organic matter content (1.8%). This could be one of the reasons for soil 3 to be more fertile, producing greater agricultural yield while soil 4 being less fertile showing minimal agricultural yield. Soils with pH in alkaline range hinder the uptake of water, which consequentially elevates the sodium content and adversely affects the underlying biochemical processes required for the sustainability of organic content. The sand content in soil 4 was found highest among all soil samples (61%) while the clay content was lowest (6.3%). Textural aspects are imperative and one of the decisive factors for governing the rate of degradation of pesticide. Based on the textural analysis, Bahawalpur and Swabi soil was found to be sandy, Mianwali soil was found to be clay in nature and Pasni soil was sandy loam. Various types of textural classes display the potential of soils variability towards degradation. Soils were also tested for their electrical conductivity. A higher EC value depicts greater salinity of soil, which impedes the water uptake and pose the peril of soil's salinity hazard.

3.2 Biodegradation experiments

Fungal strains: *Aspergillus niger* and *Penicillium chrysogenum* were added in the soil samples and spiked with BSM for bioremediation experiments. BSM was monitored as to whether or not it acts as a nutrition source for the fungi. A constant temperature (27±2°C) was provided to the samples for optimal fungal growth. No effect of temperature change on fungal cells was taken into account since a constant temperature was maintained at a constant during the experiment.

After 21 days, soil 3 displayed highest percentage of BSM degradation. Soil 3 exhibited 95% of dissipation by *Aspergillus niger* while the least reduction was seen in soil 4 (57%). Similarly, the degradation rate by *Penicillium chrysogenum* was highest in soil 3 (71%) and lowest in soil 4 (50%). These varying degradation trends were observed due to the relative percentages of OM present in the soil samples. Degradation rate of BSM was observed to be influenced by soil physicochemical properties. The presence of higher organic matter in soil 3 rendered it to be more favourable for degradation. OM provided better conditions for the fungal strains to strive in soil and convert BSM to its metabolites through the action of its enzymes, such as hydrolases and peroxidases [29,30]. Another factor playing a crucial role in the high rate of degradation in soil 3 was its low pH value as compared with rest of the soil samples. As a consequence of low pH, it exhibited a greater degree of degradation. It was observed that pH and the rate of degradation are inversely proportional. The lowest rate of degradation observed in soil 4 can be attributed to the least amount of OM (1.8%) as well as its highest pH (8.6). Soil 4 displayed a considerably higher amount of heavy metals present in it as compared to the other soils. Microbes including fungi have been found to degrade other molecules as well. The presence of heavy metals affects degradation rate of BSM as it may result in reduced or unavailability for degradation. In case of soil 4, the least degradation can be attributed to lowest amount of OM, highest pH and the presence of heavy metals (Table 2; Table 3). The increase in fungal cells was observed throughout the experiment and calculated by a haemocytometer. The fungal cells of *A. niger* and *P. chrysogenum* increased from $6.3x10^5$ and $6x10^5$ viable fungal spores mL^{-1} to $12.8x10^5$ and $11.4 x10^5$ viable fungal spores mL^{-1} respectively.

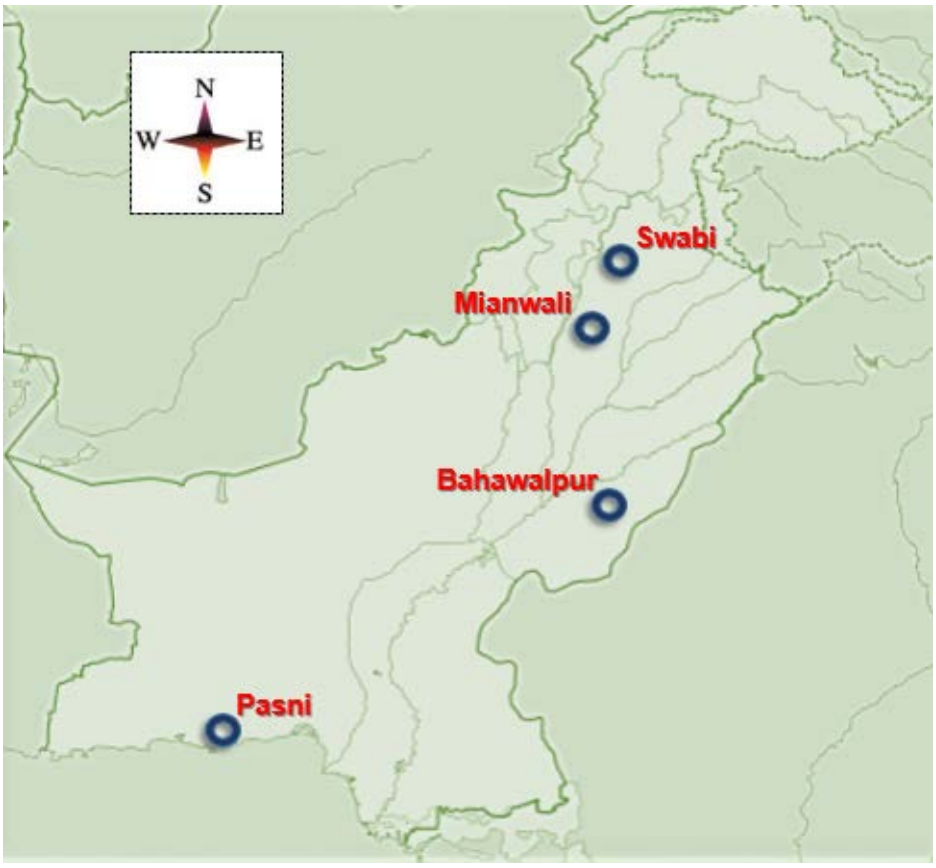

Figure 1: Map exhibiting the soil locations for transformative studies on chemical BSM.

Table 2: Biodegradation of Bensulfuron-methyl by *Aspergillus niger.*

Soil	Percentage degradation (D_t) (%)			ln[C] of pesticides concentration			Slope (k) (1/s)	Half-life $T_{1/2}$ (Days)
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21		
1	3	32	87	0.55	0.23	-4.66	-0.45	0.52
2	1	13	64	0.33	-4.92	-5.94	-1.75	1.54
3	27	46	95	-0.03	-4.03	-5.94	-1.33	0.39
4	20	21	57	1.72	0.35	-4.49	-0.10	6.93

Table 3: Biodegradation of Bensulfuron-methyl by *Penicillium chrysogenum* .

Soil	Percentage degradation (D_t) (%)			ln[C] of pesticides concentration			Slope (k) (1/s)	Half-life $T_{1/2}$ (Days)
	Day 7	Day 14	Day 21	Day 7	Day 14	Day 21		
1	25	45	68	0.68	-2.63	-5.52	-0.72	0.9
2	26	36	57	-2.08	-4.24	-4.93	-0.32	2.1
3	11	37	71	-3.16	-3.97	-4.69	-1.10	0.6
4	21	38	50	-3.86	-4.02	-4.32	-0.27	2.5

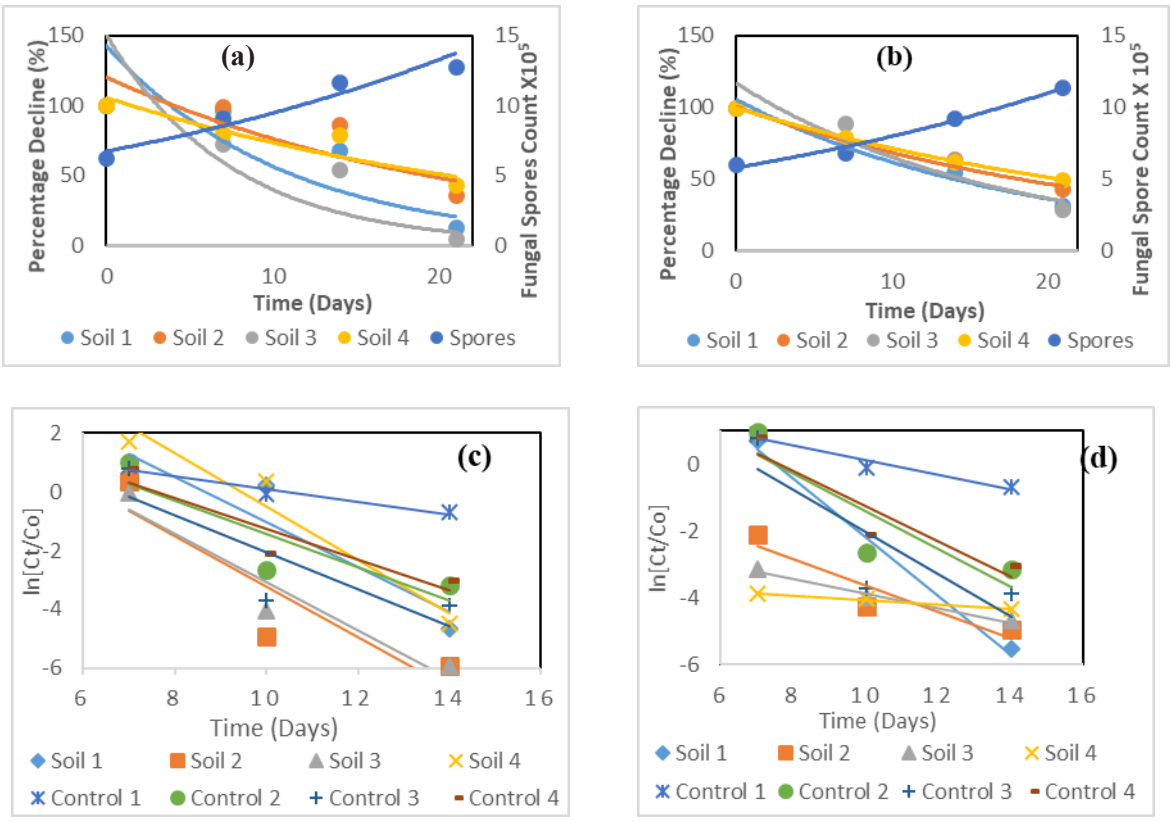

Figure 2: Biodegradation of BSM in soil samples (a) Percentage decrease in BSM concentration by *A. niger*. (b) Percentage decrease in BSM concentration by *Penicillium chrysogenum*. (c) Linear plot of ln[Ct/Co] for BSM by *A. niger*. (d) Linear plot of ln[Ct/Co] for BSM by *Penicillium chrysogenum*.

The plots of percentage degradation (D_t) versus time gave an exponential curve indicating that the decomposition of Bensulfuron-methyl followed first order reaction kinetics (Figure 1). In table 2 and 3, the percentage degradation (D_t) discernibly showed very less degradation at day 7. After that, an increase in degradation was detected on days 14 and 21. The low rate of degradation on the 7th day can be due to the lag phase of the microbes in the medium. The fungal spores take some time to be acquainted with the new environment provided, causing a delay in the decomposition process of BSM. The first order reaction kinetics equation was followed to determine the rate constant of degradation (k) for all the soil samples.

The range of biodegradation trends obtained was attributable to different pH values of the soil samples. Soils with higher pH values showed less rate of degradation than the soils with a lower pH value. This is because a higher mobility of BSM is observed in soils with a higher pH value, consequentially making the pesticide unavailable for the fungi to consume; hence, a faster rate of BSM reduction was observed in acidic soils. The BSM molecule tends to be more dissociated in soils with higher pH thus becoming polar. This provides less probability for the molecule to be available for decomposition by fungal cells as its mobility increases [31]. In Figure 1(a) and 1(b), an exponential increase in fungal spore count can be seen along with a declining trend of pesticide, Bensulfuron-methyl, in all soil samples. This confirms that the fungal spores are consuming the pesticides as their nutrition source and thus reproducing. In Figure 1(c) and 1(d), the declining trend lines of control samples can be seen giving a lesser slope. It can be visualized in the plots that the decline of BSM in control samples (containing only pesticide in soils with no fungal cells) is very less as compared to the inoculated samples. This slight decrease is due to either chemical hydrolysis or pesticide adsorption.

Analysis by GC-MS required no additional clean-up after extraction of BSM from the samples. Bensulfuron-methyl was detected in the standard solution at Rt 12.7 min in the total ion chromatogram. Several metabolites were formed as a result of BSM decomposition by fungi. The results of GC-MS were analysed and compared with the online NIST library. This study of the transformation products of BSM provided the information about how

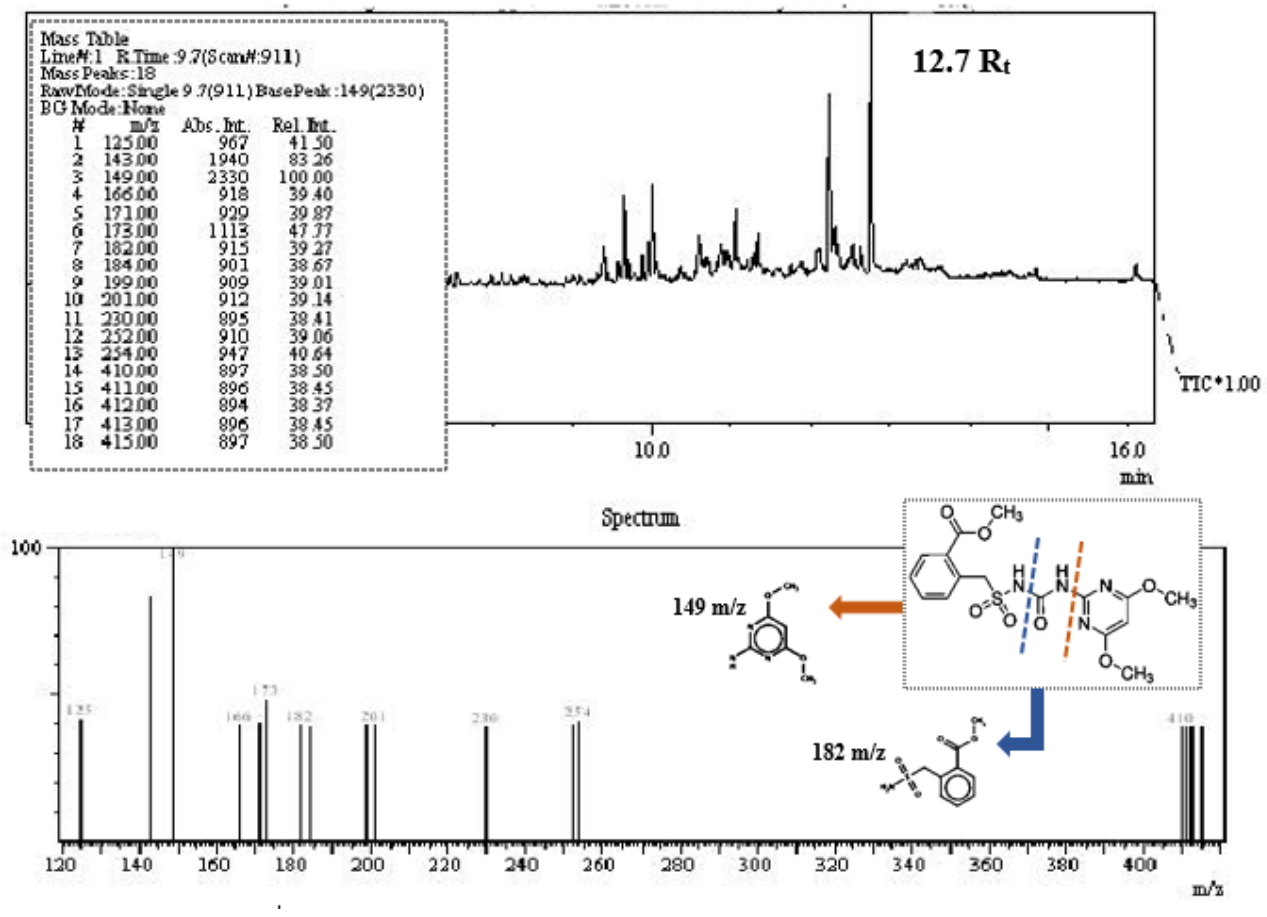

Figure 3: GC-MS chromatogram of Bensulfuron-methyl main peak at 12.7 R_t. Inset: Mass table of BSM metabolites. An m/z spectrum with main degradation products of BSM at 149 and 182 m/z.

Table 4: Ions selected for SCAN with their retention times and relative abundances for BSM molecular weight 410.

Ions	Relative abundance	Retention times m/z
Base peak $[MH]^+$	100	149
[M+H]	42.9	411
[M+2H]	43.2	412

BSM changed structurally through the action of fungal strains in the soil samples (Figure 2). According to SCAN mode, the major transformation products were obtained at m/z 149, 182, 411 and 412 [32] (Table 4). The SCAN mode highly increased the sensitivity throughout the chromatographic run [33]. The profuse daughter ion for BSM was observed to have the highest quantitative sensitivity. The SCAN mode provided affirmatory evidence about the transition products of BSM. The SCAN GC-MS has been used previously for several environmental and medical analysis. The weakest metabolite observed at the m/z chromatogram could be used to determine the lowest level of detection of BSM [34]. The sulfonylurea bridge of BSM was broken down to form pyrimidinamine [149 m/z] and benzylsulfonamide [182 m/z] [35]. BSM molecules can interact with fungal cells in a variety of processes including bioaccumulation, biosorption or biotransformation (Figure 3).

Aspergillus niger is an active degrading fungal specie, responsible for dissipation of multiple herbicides. Sharma et al. reported the biotransformation of pesticide chlorimuron-ethyl by *A. niger* by two routes, sulfonylurea bond cleavage and scission of sulfonylamide link [36]. Other herbicides commonly degraded by *A. niger* include, nicosulfuron, metsulfuron-methyl, chlorsulfuron [37-39]. Chlorimuron-ethyl, a sulfonylurea herbicide, displayed 81% degradation by *Pseudomonas sp.* [40]. In another investigation, three sulfonylurea herbicides, rimsulfuron, triasulfuron and primsulfuron were degraded in microbially active soils, which displayed shorter half-lives than in sterile soils [41].

Table 5: Mean recovery concentration and standard deviation of residuals.

Days	Mean recovery concentration (mg)	Sample S.D.[1]	R.S.D.%[2]
7	2.400	2.150	89
14	0.650	0.750	114
21	0.006	0.004	59

[1] S.D.= standard deviation
[2] R.S.D.= relative standard deviation

Table 6: Linear regression analysis and analysis of variance on Bensulfuron-methyl biodegradation by fungi.

Fungal strains	Soil samples	P-value	Linear regression equation	R^2
Aspergillus niger	Soil 1	0.03	y = 0.30x + 27	0.9
	Soil 2	0.04	y = 0.70x + 30	0.9
	Soil 3	0.02	y = 0.50x + 10	0.8
	Soil 4	0.05	y = 0.25x + 44	0.9
Penicillium chrysogenum	Soil 1	0.01	y = 0.93x + 13	0.9
	Soil 2	0.06	y = 0.28x + 25	0.8
	Soil 3	0.05	y = 0.50x +19	0.9
	Soil 4	0.004	y = 0.22x + 54	0.9

3.3 Statistical evaluation

The means of residual quantities acquired were calculated. The standard deviations and relative standard deviations (R.S.D.) were evaluated (Table 5). R.S.D. between different soils was high at all extractions because of the distinct differences among the recovery rate of each soil due to the varying physical and chemical characteristics. Furthermore, ANOVA statistical tool was applied on the degradation percentage (D_t) for both the strains indicating p value to be < 0.05 in both cases, thus proving the significance of the degradation results. The R^2 values for all soils for both strains were close to 1 indicating the significance of results (Table 6).

3.4 Hydrolysis experiments

3.4.1 Effect of pH

The process of degradation is not only a result of microbial action but also a consequence of chemical degradation. During hydrolysis, the decrease in pH is the main factor governing the rate of decomposition of BSM [42]. When fungal cells initially attack the pesticide, it lowers down the pH. Hence, further losses occur through the hydrolysis of BSM in media [43].

Chemical hydrolysis experiments were also performed on soil and corresponding water samples. pH of the water samples was adjusted according to the corresponding soils. Highest degradation rate was seen in soil 3 (48%). Although the reduction is quite less than fungal degradation (95%), it is still a decent degradation percentage through hydrolysis. During the process of hydrolysis, the carbonyl carbon bridge is cleaved by water, which releases CO_2 creating sulphonamide and heterocyclic amine. The amine further converts to metabolites [44]. The degradation through hydrolysis can be observed in Figure 4. The rate of hydrolysis in water samples did not deviate much from that observed in soil samples. This was because the pH of water samples was attuned with that of the corresponding soils. The highest hydrolysis rate observed in soil 3 was also because of the lowest pH [45]. Even in case of fungal degradation, the increased and rapid decomposition was due to the assisted hydrolysis of the pesticide.

3.4.2 Effect of temperature

Effect of temperature change was also assessed in order to observe the change in the rate of degradation. All the vials were initially placed at a reduced temperature (4°C) for two days. After two days, UV analysis was done following liquid-liquid extraction. No change in the UV absorbance was observed indicating that same amount of Bensulfuron-methyl was still present in all the samples [46]. The temperature was then increased to 15°C. The samples were kept at this temperature for another two days followed by UV analysis. A slight decrease in the absorbance peak was detected by the UV-vis spectrophotometer. This depicted that the herbicide decomposed to some extent through chemical break down. The temperature was again increased to an optimum 27°C for two days. UV analysis exhibited a sharp decline in the concentration of BSM (Figure 5). Briefly, it can be stated from the obtained results that lower temperatures did not encourage BSM degradation while elevated temperature accelerated its decomposition by breaking down the sulfonylurea bridge in its structure [47]. Increased temperature at an optimum range of 27°C was ideal for BSM hydrolysis.

Generally, sulfonylurea herbicides display varying responses towards hydrolysis mechanism. It is mostly dependent upon the chemical structure of the herbicide

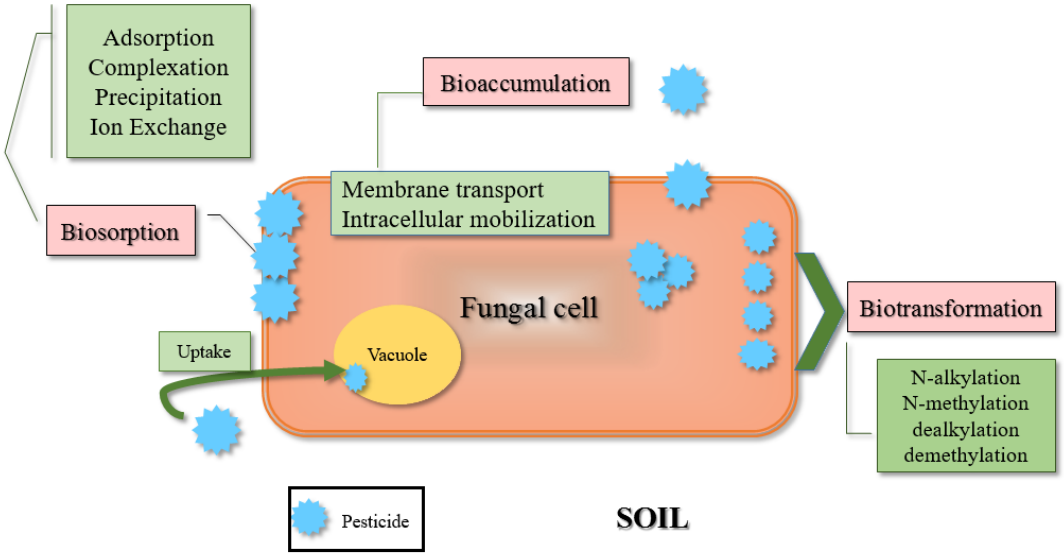

Figure 4: Possible interactions of pesticide with a fungal cell in soil.

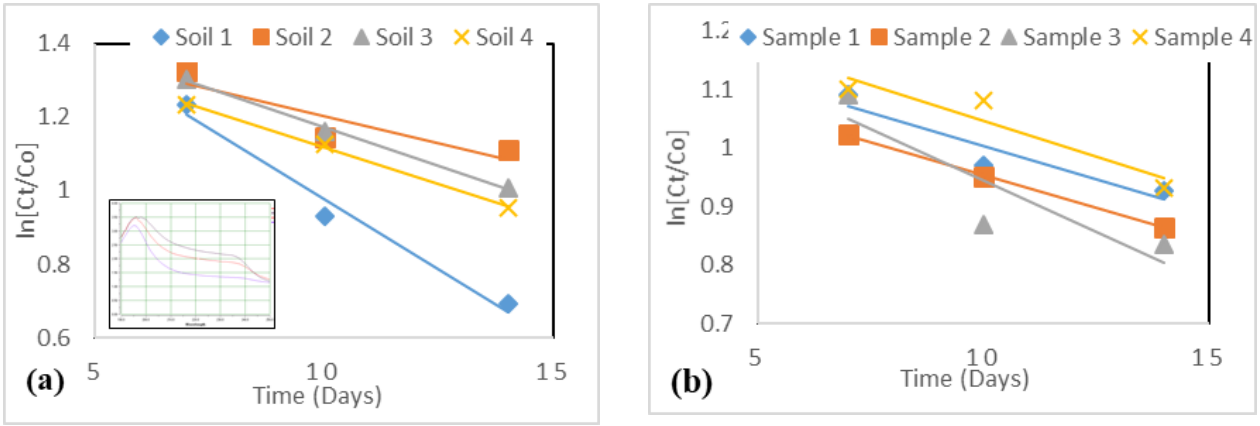

Figure 5: Linear plots of log of concentration of Bensulfuron-methyl hydrolysis with time (days). (a) Hydrolysis in soil samples. (b) Hydrolysis in water with pH corresponding to that of soil samples.

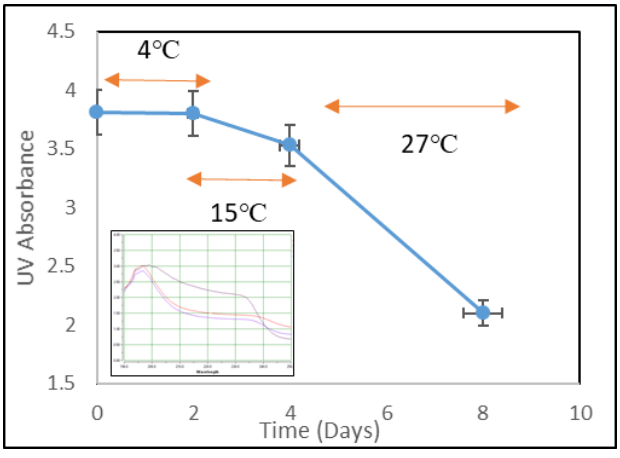

Figure 6: Temperature effect on hydrolysis rate in soil sample 3.

and temperature/pH of the environment. Dinelli et al. also displayed a varying response of primsulfuron, rimsulfuron and thiensulfuron towards hydrolytic degradation. Some herbicides displayed rapid degradation while some exhibited extended half-lives of 49-399 days [48]. The dissipation rates of all the herbicides depended upon range of temperature and pH of the medium.

4 Conclusion

Bensulfuron-methyl was studied for its degradation behaviour in four soils through the use of common soil borne fungal strains. Following the solid-liquid extraction

of Bensulfuron-methyl in four soil samples, it was observed that pH factor and temperature are highly imperative for the rate of degradation. Fungal strains: *Aspergillus niger* and *Penicllium chrysogenum* showed high rates of BSM degradation. Acidic soils showed more rapid degradation than alkaline soils. Similarly, an optimal temperature of 27°C was found ideal for degradation. Although these studies were carried out in laboratory under artificial conditions, they will be crucial to predict the effect of degradation and the formation of the transformation products in actual field conditions. Fungal degradation and chemical hydrolysis are dominant modes of decomposition of Bensulfuron-methyl in soils and liquid media. However, further investigation is needed to find the mechanisms of fungal enzymes and its mode of action to assess the degradation trends for various chemicals. The results provide a critical insight into bioremediation of BSM contaminated soils.

Conflict of interest: Authors declare no conflict of interest.

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