Effect of Chemical Weed Control on Soil Bio-Chemical Indices- A Review

Divya Singh[1], Atish Yadav[2*], Shivendra Singh[3], Adesh Kumar[4], Abhishek Mishra[5]

[1,2]Department of Agronomy, Acharya Narendra Deva University of Agriculture & Technology, Ayodhya, (UP), India
[3,4,5]Department of Agronomy, Chandra Shekhar Azad University of Agriculture & Technology, Ayodhya, (UP), India
*Corresponding Author

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Abstract— *The assessment and monitoring of soil life and soil health can be used to develop more sustainable and productive farming systems. Hence, the consequence of herbicide application on soil health is always a concern for the research community. In view of this, the findings available from India in respect to the impact of herbicides on the non-target organisms and important soil bio-chemical processes are reviewed in this paper. There is great variation among the reports showing short term transient depressing to non-inhibitory or even stimulatory effects of herbicides on total soil microbial count and different soil bio-chemical indices. The impact differed depending upon the soil type, experimental conditions, herbicide in question and its dose, and the sensitivity of the non-target species or strains. No severe ill effect on soil flora, soil bio-chemical indices and soil fauna has been observed so far at recommended dose of herbicide under field conditions. However, the available information is based on the short term experiments and there is need to develop data base on long-term field application basis. The paper concludes with some suggested areas for future research requiring urgent attention*.

Keywords— Actinomycetes, Ammonification, Bacteria, Earthworms, Fungi, Herbicides, India, Nematodes, Nitrification, Nitrogen fixation, Soil enzymes.

I. INTRODUCTION

Soils contain microorganisms viz. bacteria, fungi, yeasts, photosynthetic organisms including algae and macroorganisms such as protozoa, nematodes, mites, springtails, spiders, insects and earthworms. The functions of this complex array of biota are diverse, and include residue decomposition, nutrient storage and release, soil structure and stability, resistance against disease and degradation or immobilisation of soil pollutants. A minimum of species is necessary to carry out essential tasks. It is believed that high biodiversity leads to a higher soil functional stability and thereby, a greater capacity to recover from perturbation and maintaining environmental sustainability.

Weed control in agricultural and non-agricultural lands is rapidly shifting towards chemical methods because of its time, labour and cost advantages. Although herbicides are meant for plants, possibility of a direct effect on other organisms can not be ruled out as a number of basic and universal biochemical processes essential for all forms of life are alike. Direct impacts on sensitive organisms can occur when the chemical reaches the soil due to targeted deposition of pre-emergent herbicides, or through unintentional deposition from spray and spray drift, dripping from plant material, and contaminated plant material falling to the soil.

A decrease in the population of sensitive species may cause an increase in the population of resistant soil microorganisms due to relatively lesser competition. Thus, their application may have impacts on organisms that benefit the wider agro-ecosystem. Such concerns from the research community and general population were well documented in June 1992 at the United Nations Conference on the Environment and Development (UNCED) in Rio de Janeiro, which is referred to as the Convention on Biological Diversity.

In field conditions, herbicides may also greatly influence soil biota populations indirectly by their effects on vegetation which provide habitat and food for many of them. The soil organisms may respond differentially due to the changes in vegetation rather than to direct herbicide effect (Grossbard and Davies 1976, Haugland 1994). Influence of herbicides on soil biota

population and agriculturally important soil bio-chemical processes have been reviewed in this paper in light of the findings reported from India.

II. EFFECT OF HERBICIDES ON SOIL MICROFLORA

The total microbial count is the direct measurement of qualitative change appearing after herbicide treatments. Highly contrasting reports are available in the literature in respect to the side effect of herbicides on soil micro-flora. The observations varied from adverse to no effect or even stimulatory effect on microbial growth after herbicide application. Reports indicate the adverse effect of herbicides on selected species of microorganisms in pure culture and many a times at the higher concentration level that is unlikely to occur in the actual field condition at recommended rates of application. No serious or prolonged effect of herbicides on total count of soil microflora was reported. No adverse effect of propanil, nitrofen, prometryne, 2,4-D, bentazon and butachlor application at recommended rates on soil populations of bacteria, fungi and actinomycetes was observed in rice in West Bengal (Mukhopadhyay 1980). Raut et al. (1997) observed that except for a slight initial suppressing effect, butachlor stimulated the microbial population of rice rhizosphere in a Delhi soil. Similarly, at field rate, a short term transient or stimulatory effect of propanil, butachlor, molinate and nitrofen on microbial population in transplanted rice soil was reported by Shetty (1977). Pendimethalin steadily increased the total population of bacteria, fungi and actinomycetes in soil under cotton after a short lag phase during the crop growth period. However, after harvest the soil microorganisms were affected by the pendimethalin residue in soil (Balasubramanian and Sankaran 2001). Generally in field condition, a short time initial depressive effect is followed by an increase in the total bacterial number to the normal level. This delayed stimulation is caused by the adaptation time of the bacteria. Initial depression could be due to the adverse impact on susceptible strains and subsequent increase in the growth rate of the relatively resistant strains with due course of time. The subsequent increase in bacterial number could also be due to the increase in the environment of nutrients that come from weeds killed by the treatment. It can also be explained by the utilization of the herbicides as substrates by the resistant strains.

The amount of herbicide coming into physical contact is of great importance to side effects. In the field condition most of the herbicides do not penetrate more than few millimeters into the soil. Thus, there is rarely total exposure of soil microorganisms to a biologically active concentration of a herbicide. A change in species composition of soil microorganisms may occur after herbicide application but elimination of a single species is very unlikely because the nature will try to restore the former equilibrium quickly (Greaves and Malkomes 1980). However, herbicide-induced shifts in microbial composition may occur even if diversity indices among treatments remain same (Lupwayi et al. 2004). VAM fungi are beneficial and live in association with plant roots. The dissimilarity in the results obtained with herbicides belonging to the same group of chemicals, or even with the same herbicide makes it more difficult to generalize the effect of herbicides on VAM. A herbicide may inhibit the VA colonization by some individual strains but not by others (Dhen et al. 1990, Dodd and Jeffries 1989), clearly showing the direct impact of herbicide on fungus. Oxyfluorfen reduced VAM colonization and spore production in tomato (Abha Mishra and Mishra 1999). While, 2,4-D enhanced both the percentage of infection and number of mycorrhizal propagules in Sesbania grandiflora and Albizia lebbeck (Kumar et al. 1999). The species or even the cultivar of host plants can influence the impact on the herbicides on VAM. The reduction in VAM colonization and spore production in tomato due to oxyfluorfen application varied among the different tomato cultivars tested (Abha Mishra and Mishra 1999). The herbicides, besides a probable direct chemical effect on VAM, do kill the plants and reduce the living food source of the VAM fungi. This may in turn also influence VAM growth and survival.

III. EFFECT ON IMPORTANT SOIL BIOCHEMICAL PROCESSES

Determination of qualitative changes of the huge populations of thousands of species following herbicide application is impossible. There is no universally accepted indicator till date to study the effect of herbicide on soil microflora. The important biochemical processes from agricultural and environmental perspectives are mostly mediated by a group of microbial species and strains. Since sensitivity to a given herbicide varies greatly among the different microbial species and strains, the information related to the side effect of herbicides on the agriculturally important microbial processes as a whole are of greater significance than the observations about a given species or strains.

IV. MICROBIAL ACTIVITY

Due to the technical and practical limitations, the total count data do not distinguish between inactive microorganisms and those really active in soil. Measurement of the activity of the soil microflora provides indexes of the biological state of the soils and hence the soil fertility. Assessment of the enzymes present in soils offers potential as an integrative index of the soil's biological status. Dehydrogenase activity is generally used as an index of metabolic activity of the microbial population in soil.

Except a slight depression initially, butachlor at field rate was generally non-inhibitory in its effect on dehydrogenase activity in rice on a Vertisol (Rao and Saroja Raman 1998). While an initial stimulation in dehydrogenase activity following fluchloralin, butachlor, oxyfluorfen and 2,4-D application was reported by Shukla (1997). Baruah and Mishra (1986) reported that the herbicides 2,4-D, butachlor or oxyfluorfen at the manufacturer's recommended rates to a paddy soil initially stimulated but subsequently (after 7 days) inhibited dehydrogenase activity. Similarly, diuron at 10-100 ppm stimulated dehydrogenase activity in black, laterite and coastal saline soils of India (Sarawad 1987). Carbon dioxide evolution is another important indicator of overall microbial growth and activity in soil. Stimulation in carbon dioxide evolution was recorded due to the application of 2,4-D, butachlor or oxyfluorfen at the manufacturer's recommended rates to a paddy soil (Baruah and Mishra 1986). No adverse effect of butachlor application to rice soil was also reported by Mukhopadhyay (1980). Nitrofen at 100 times the normal rate increased bacterial numbers, dehydrogenase activity and respiration of black clay and red sandy soils (Kale and Raghu 1989). Application of dinitroaniline herbicide pendimethalin significantly inhibited the soil respiratory activities and dehydrogenase enzyme in the rhizosphere of wheat (Shetty and Magu 1997). The extent of inhibitory effect of herbicide on soil dehydrogenase activity and short-term respiration depended on soil type, plant growth and sampling time (Malkomes 1988).

V. AMMONIFICATION

Ammonification of organic form of nitrogen is carried out by wide groups of soil micro-organisms. In an incubation study simulating the flooded condition, Shukla and Mishra (1997) observed that addition of butachlor at 6 mg/kg dose did not have any remarkable effect on ammonification of urea. No significant effect of fluchloralin, butachlor, oxyfluorfen and 2,4-D application on urease activity was observed in a sandy loam soil (Shukla 1997). Similarly, diuron did not inhibit ammonification in black, laterite and coastal saline soils of India even when applied at 100 mg/kg rate (Sarawad 1987). The effect of herbicide may also vary depending upon the soil and environmental factors. The field rate of 2,4-D stimulated ammonification in red sandy clay loam soil but there was no significant effect in black cotton clay soils. While at 5 times of field rate, 2,4-D depressed ammonification in both soils (Deshmukh and Shrikhande 1975).

VI. NITRIFICATION

Unlike ammonification process nitrification is carried out by a very small group of soil bacteria, mainly Nitrosomonas and Nitrobacter. Hence, any probable impact of herbicides on this group of bacteria is of great concern from soil fertility point of view. Moreover, both Nitrosomonas and Nitrobacter are compulsorily needed to complete the oxidation of ammonium–N to nitrate–N, the most preferred form of N for plants. In a laboratory studies, herbicides at field rates generally showed a temporary depressing effect on nitrification that recovered within a short period of time and nitrification proceeds as normal. No marked effect on nitrification of NH4 -N at pH 6.8, but a slight depression at pH 4.9 at 30o C was recorded due to addition of butachlor in soil under laboratory condition (Shukla and Mishra 1997). However in the actual field condition, application of butachlor at 2 kg/ha significantly augmented the availability of mineral N, i.e. exchangeable NH+ 4 and soluble NO3 , in the rhizosphere soil of rice (Debnath et al. 2002a). This showed that there was acceleration of both ammonification and nitrification by rhizosphere microflora resulting in higher release of mineral nitrogen in soil. Diuron at 10-100 mg/ kg, inhibited nitrification in black, laterite and coastal saline soils of India; the inhibitory effect increased with increasing pesticide concentration (Sarawad 1987). Hardly any report is available about any intense adverse effect of herbicides on nitrification in the field situation. Reports available so far indicate that herbicides are generally not harmful on nitrification, rather beneficial at times. Nitrification process was stimulated by 2,4-D-sodium for 2 weeks in a black cotton clay soil but for 1 week in a red sandy clay loam soil; whereas, no stimulation in nitrification was noticed in case of 2,4-D-ester (Deshmukh and Shrikhande 1975).

VII. DENITRIFICATION

It is an important component of soil nitrogen cycle and in Indian context, where available soil N is a constraint for crop growth, it may be considered as a deleterious soil biochemical process from soil fertility point of view. The impact of the herbicides on the growth and activity of the microorganisms related to the denitrification process under Indian agro-climatic situation is being overlooked by the research community. There is severe lack of information in this area so far and it requires attention especially in light of the reports from elsewhere (e.g. Tu 1996, Tenuta and Beauchamp 1996) indicating the stimulatory effect of several herbicides on the denitrification process.

VIII. NITROGEN FIXATION

Nitrogen fixation by symbiotic organisms associated with legumes is of immense importance throughout the world. The adverse impact of herbicides on survival and growth of Rhizobia is observed beyond a threshold concentration which depends

on the type and concentration K.K. Barman and Jay G. Varshney 13 of herbicide used and also on the species/strain of the Rhizobia studied. Mostly the adverse impact is recorded when the herbicide is added in excess of field recommended rates. At field rates of addition most herbicides are unlikely to have much effect on rhizobial growth. Nitrofen stimulated Rhizobium in pure culture (Kale and Raghu 1989). Singh et al. (1978) reported that few strains of Rhizobium leguminosarum were more resistant to butachlor than few strains of cowpea Rhizobium or R. japonicum and Rhizobium tolerated higher concentrations of butachlor than the blue-green algae. However, compared to mechanical or manual weeding, a decrease in nodulation in legumes due to herbicide application is often reported in the literature. Lentil showed adverse impact to the application of oxyfluorfen, linuron, metribuzin, and oxadiazon in terms of nodulation and nitrogenase activity (Sandhu 1991). Pendimethalin and fluchloralin also showed toxic effect to the nodulation in lentil compared to the hand weeding treatment (Yadav et al. 1990). Similarly, a decrease in the nodulation and nitrogenase activity in pea was caused by methabenzthiazuron, linuron and pendimethalin application (Gurcharan Singh et al. 1994). However, the toxic effect of herbicides on nodulation generally disappears with time. For example, fluchloralin, metribuzin and oxadiazon showed toxic effect on soybean nodulation at 25 DAS, but the adverse effect disappeared by 50 DAS (Jain et al. 1990). No harmful effect of imazethapyr on nodulation in soybean was observed by Billore et al. (1999). Similarly, at field rate of application to soybean, fluchloralin in combination with the rhizobial culture and (or) plant growth promoting rhizobacteria showed better nodulation and nitrogenase activity compared to the inoculated but no pesticide treatment and the uninoculated control (Murali Gopal 2002). It may be noted that herbicides may affect legume-Rhizobium symbiosis in different ways by reducing survival or growth of Rhizobia by inhibiting the nodulation process by causing abnormalities in plant growth and metabolism; or by influencing nitrogen fixation.

A number of reports indicate the adverse impact of herbicides under laboratory conditions, however no serious effect of herbicide application at recommended dose on free living N-fixers has been reported under field conditions. Toxic effect of butachlor (at 2 µg/g) on Azospirillum population in alluvial and acid sulphate saline Pokkali soils was reported by Jena et al. (1987). However, Rai (1985) isolated butachlor-resistant strains of Azospirillum brasilense from roots of rice. Patnaik and Rao (1994) reported about a substantial stimulation in nitrogenase activity of Azospirillum isolated from 2,4-D amended rice rhizosphere soils, following exposure to 2,4-D at concentration up to 5 ppm under normal fixing conditions. Addition of ammonium-N significantly reduced its nitrogenase activity, but the toxic effects of combined nitrogen were alleviated in the presence of 2,4-D. An increase in root-associated aerobic and microaerophilic N2 fixing bacteria and stimulation in nitrogen fixation activity of young barley seedlings by pendimethalin at field rate in a neutral alluvial loam soil was reported by Jayanta Saha et al. (1991). The stimulatory response, however, declined with age of seedlings and higher concentration of the herbicide. Azotobacter vinelandii and Azospirillum lipoferum isolated by these workers from the pendimethalin-treated barley rhizosphere showed in vitro tolerance to high concentrations of the herbicide in N-free media; and the Azotobacter isolate utilized pendimethalin as a C source to fix N2 in pure culture. The property of pendimethalin utilization for N2 fixation was also exhibited by Azotobacter chroococcum (Jayanta Saha et al. 1991). It was observed in dark laboratory conditions that the soil bacterium A. chroococcum can effectively degrade pendimethalin (Kole et al. 1994). Unlike the total population of bacteria and fungi, an increase in actinomycete and Azotobacter population by pendimethalin at 1.5 ppm concentration was recorded in a sandy loam soil (Shetty and Magu 1996). A. chroococcum also showed the ability to utilize the herbicide 2,4-D and its degradation products, p-chlorophenoxyacetic acid and p-chlorophenol as sole carbon source, and showed an increase in oxygen uptake and stimulation in nitrogenase activity in presence of the chloroaromatics (Balajee and Mahadevan 1990). The nitrogenase activity in four A. chroococcum strains isolated from agricultural soil, enriched with 2,4-D, remained unaffected up to 50 ppm of 2,4-D in liquid medium (Gahlot and Narula 1996). Seed inoculation with A. chroococcum increased grain and straw yield, and also reduced the phytotoxic effects of 2,4-D on wheat on a sandy loam soil (Ajit Singh et al. 1997). No adverse effect of diclofopmethyl application up to twice the recommended dose on the Azotobacter population was noticed in the soil of a wheat field at harvest (Singh et al. 1996). While, nitrofen inhibited A. chroococcum in pure culture (Kale and Raghu 1989). Application of butachlor at 2 kg/ha, significantly augmented the proliferation of aerobic non-symbiotic N2 fixing bacteria and hence the non-symbiotic N2 fixing capacity of the rhizosphere soil of rice (Debnath et al. 2002a).

Under laboratory condition, application of butachlor reduced populations of anaerobic nitrogen fixers in a nonflooded alluvial soil, but stimulated its population in an acid sulphate saline Pokkali soil under a similar water regime (Jena et al. 1987). However, in submerged condition, butachlor stimulated nitrogen fixation in the alluvial, lateritic Impact of herbicides on soil environment 14 and acid sulfate soils (Jena et al. 1990).

Algal growth is sensitive to herbicide application but the sensitivity varies among the different species and also depending on the herbicide. Likhitkar and Tarar (1996) reported that increasing butachlor concentrations gradually reduced the nitrogen fixation by Nostoc commune and N. muscorum but did not retard cyanobacterial activity at the normal recommended field

application dose and can safely be used with these cyanobacteria. N. muscorum was more tolerant than N. commune to butachlor. Kashyap and Pandey (1982) reported that butachlor at low concentrations (0.05 µg/ml) had stimulatory effects on Anabaena doliolum, but completely inhibited its growth at 20µg/ml. However, the increased concentration of butachlor did not have any adverse effect on Anabaena sphaerica, rather it accelerated the algal contribution in terms of biomass and nitrogen fixation (Suseela 2001). Low concentrations of butachlor significantly increased heterocyst spacing in Anabaena doliolum. The nitrogenfixing ability of A. doliolum and Nostoc muscorum was not affected by butachlor but was reduced at the higher concentrations (Singh et al. 1978).

IX. DECOMPOSITION OF ORGANIC MATTER:

A favourable effect of glyphosate and 2,4-D on growth and activity of several strains of cellulolytic bacteria, namely, *Cellulomonas, Pseudomonas, Clostridium, Polyangium, Clonothrix, Sporocytophaga, and fungi, Aspergillus syndowii and Fusarium oxysporum*, isolated from tea plantations, was reported by Bora and Bezbaruah (1992). The test strains degraded the various weed litter (e.g. *Cynodon dactylon, Glyceria maxima, and Legurus ovatus*) sprayed with glyphosate and 2,4-D at faster rate than the untreated counterparts. Unlike the herbicides glyphosate and 2,4-D that increased the population of cellulolytic strains, dalapon and paraquat reduced it in the soil of a tea plantation (Balamani Bezbaruah et al. 1995).

X. PHOSPHORUS AVAILABILITY:

Oxyfluorfen has been shown to increase phosphorus availability in rhizosphere soil (Das et al. 2003). Phosphatase [phosphoric monoester hydrolase] activity was increased by fluchloralin, butachlor and oxyfluorfen, but was reduced by 2,4-D (Shukla 1997). Its application significantly augmented the proliferation of phosphatesolubilizing microorganisms in the rhizosphere soil of wetland rice, and there was a significant positive correlation between the population of phosphate solubilizing microorganisms and phosphate solubilizing capacity in the soil (Debnath et al. 2002b). Application of dinitroaniline herbicide pendimethalin significantly inhibited the soil phosphatase enzyme in the rhizosphere of wheat (Shetty and Magu 1997). In vitro alkaline phosphatase activity in Anabaena under glyphosate treatment showed increase in enzyme activity compared with the untreated control (Ravi and Balakumar 1998).

XI. EFFECT ON SOIL FAUNA:

The soil fauna plays an important part in the decomposition of litter in soil, they may indirectly increase aeration and drainage in the soil while feeding on decayed woods, contribute to the formation of humus in association with soil bacteria; and hence considered to be beneficial in relation to the structure and fertility of soil. On the other hand there are some injurious groups of soil fauna, e.g. parasitic nematode.

Nematodes occupy an important place in microscopic life and belong to the soil microfauna group. The interactions of herbicides with nematodes of higher plants are generally noticed. Changes in the incidence of plant diseases may result from the application of herbicides through the effect they have on the pathogen, the host or microorganisms in the environment. Herbicides belonging to different chemical groups were found to increase or decrease nematode diseases of many plants (Trivedi 1988). In a long-term study under tea plantation, Gope and Borthakur (1991) noticed that nematodes (*Helicotylenchus, Meloidogyne, Paratylenchus and Trichodorus spp*.) population was increased by glyphosate, dalapon and simazine, while adversely affected by diuron. Swain et al. (1991) compared the application of bensulfuron-methyl, butachlor, quinclorac, thiobencarb, pretilachlor, pendimethalin, piperophos and 2,4-D at field rates with manual weeding for the control of nematode Hirschmanniella mucronata in rice. Butachlor and pretilachlor were the most toxic and resulted in the lowest nematode populations one month after application. While, application of alachlor and fluchloralin at field rates to soybean increased the soil nematode population in a deep alluvial soil (Mohammed 1987). However the effect of these herbicides varied depending upon the nematode species and the crop growth stages. Alachlor increased Longidorus spp. until crop maturity as well as Aphelenchus and Hoplolaimus during crop branching. Fluchloralin markedly increased the numbers of Tylenchorhynchus spp., especially towards the end of the growing season.

The acute toxicity of butachlor to the earthworm *Drawida willsi* was determined by Smeeta Panda et al. (2002). The 96-hour LC50 values for juvenile, immature and adult earthworms were found to be much higher than the recommended agricultural dose of butachlor. Contrary to this, Panda and Sahu (2004) reported that butachlor was toxic to earthworms at agricultural rates. A decrease in the earthworm population was observed in a rice field K.K. Barman and Jay G. Varshney 15 due to pendimethalin spray or cultural methods of weeding, but there was no difference between the two weed management practices (Mishra et al. 1996). The complete mortality of the earthworm *Eisenia fetida* was seen when directly exposed to atrazine and oxyfluorfen by filter paper, but there was no mortality when the herbicides were applied in soil. Although some physiological

and behavioural changes were observed at higher doses, both the herbicides were nontoxic in the soil at normal exposure and were relatively safe for earthworms (Chitra Srivastava 2002).

XII. CONCLUSION:

Herbicides being toxic to plants may exert some kind of impact on other life forms in soil by their direct chemical action and by changing the soil ecosystem as result of changes in vegetation cover. Overall, the experimental results showed that the population of soil microflora are stimulated or depressed by herbicides, depending upon the chemical nature, preparation, its dose, and sampling time and soil type. It is difficult to draw conclusions from such varied results on the counts of the microorganisms of the soil vis-a-vis herbicide application. However, at recommended rate of herbicide application, often a reversible change in the equilibrium of the population of micro-flora and fauna takes place in soil for a short period of time under field conditions.

It may be kept in mind that to effectively evaluate the relative effects of different agricultural practices in the long-term it is necessary to sample until the ecosystem has achieved some degree of equilibrium rather than monitoring only initial cropping cycles (Yeates *et al*. 1999). If herbicide application is to remain a viable practice in sustainable farming systems, evaluation of herbicide effects from repeated and long-term use is essential to ensure optimum nutrient availability and plant growth. However, the literature available so far is based on either laboratory experiments or short-term field experiments. Report on the basis of well-planned long-term field experiment is not available to draw any conclusion regarding the environmental implications of herbicide. Therefore, the steps should urgently be taken to generate data on long-term application basis. Changes in the many vital soil processes become visible in a long term, for example changes in soil organic C content. Some processes show "transition phenomenon", that is an impact may continue for years without any visible changes in the measured soil characteristics; and after a certain transition time the characteristics change at rapid rate. For example nitrate leaching from grasslands due to mineral nitrogen fertilization did not vary much during the initial years, but after several years it increased suddenly in spite of that the mineral N fertilization remained the same. Feasibility of such "transition phenomenon" in the microbially mediated important soil processes in respect to the soil fertility and productivity should not be ignored, especially in light of the reports showing the differential effect by the different group of microbes, even strains, to a given herbicide. Long-term experiments and data base is needed to fore see such probabilities and to derive suitable remedial measure.

Most of the information generated so far are of superficial in nature and dealt primarily about total counts. There is a dearth of information regarding the herbicide effect on the changes in microbial diversity, nitrification, denitrification, sulfur oxidation, mineralization of plant nutrients, crop residue decomposition and its consequence upon quantitative and qualitative aspect of soil organic matter equilibrium. In depth study in respect to the herbicide effect on biological nitrogen fixation is also meager. Future research is very much warranted in these directions. No serious effort has yet been made to study the dynamics of various groups of soil fauna in the fields receiving herbicide application, and it needs more attention.

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