Review *paper*

Micro-morphology of the rhizosphere with focus on plant nutrition: a review

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The paper highlights the historic background of Rhizosphere investigations focusing on Root-Soil Interface activities in terms of micro-morphology, using advanced micro-morphological investigation techniques and tools such as high-resolution microscope, scanning electron microscope, electron-probe X-ray-analyser technique, X-Ray Diffraction Technique, Thin Micro-Autoradiography using Kodak thin myler films onto thin sections with optical polish < 1.0 micron. The second part of the paper refers to a number of factorial pot experiments with French beans (*Phaseolus vulgaris* L) as test crop, involving three nitrogen sources (viz. ammonium phosphate, choline phosphate and calcium chloride) at 2 N levels (500 ppm n and 1000 ppm N), 2 initial soil pH levels adjusted at pH 7 & pH 8 and two growth (G) stages (21 days and 42 days) were conducted under a standard growth chamber condition (temperature 28^0C, light intensity 10, 000 LUX, R.H. 60%). Following dry ashing (475^0C) shoots and roots were analysed for $H_2PO_4^{-2}$ (Phospho- molybdate method (Jackson, 1964) Ca^{2+}, Mg^{2+} , $K+$,$Na+$, NH_4+ , NO_3^- , Cl^-), dithinate-citrate extractable Fe^{2+} , Mn^{2+} , Zn^{2+} (Mehra and Jackson, 1960). The analytical values of soil (rhizosphere and non-rhizosphere) and root and shoot parameters were subject to Analysis of Variance Tests (ANOVA Test) to determine significant differences of based on single factors of factorial combinations and reveal 'main effects' and interactions. The summary of results of these experiments is briefly summarized in the relevant sections of the paper.

Key words: Micro-morphology, Rhizosphere, Soil-Root Interface, Plant Nutrition, Factorial Experiment

INTRODUCTION

Definitions of Rhizosphere

The rhizosphere is defined as the narrow region (1-2 mm) that surrounds the roots of land plants. Rhizosphere activity and processes are strongly influenced by plant exudates and soil microorganisms. Plants exude various primary and secondary metabolites through the rhizoplane to the neighboring soil rhizosphere that protect the root system from soil pathogens, attract and select useful microorganisms and interact both positively and negatively with other plants, thereby promoting plant growth. The interaction between the plant rhizosphere and surrounding soil is becoming a research hotspot for chemical ecologists. Researchers are now equipped with novel technologies including Next Generation Sequencing, the use of soil profiling and microprobes for genomics, transcriptomics and metabolomics studies, and sophisticated techniques for culture of novel organisms, all of which significantly accelerate our ability to conduct informative studies in soil systems, including those addressing chemical ecology in the rhizosphere, and the role of plants and microorganisms in these interactions.

Rhizosphere is the narrow region of soil or substrate that is directly influenced by the root secretion (exudates) and associated microorganisms known as the root microbes. Lorentz Hiltner (1904) defined the rhizosphere as the area around a plant root that is predominantly inhabited by a unique population of microorganisms and postulated by the chemicals released from plant roots. Hiltner (1904) observed an abundant and preferential colonization of the soil in the vicinity of plant roots by microorganisms. He described the area immediately adjacent to the roots as the 'rhizosphere'. Since then many attempts have been made to describe and define the zone of enhanced

microbial activity with greater accuracy and this has led to the introduction of such terms as 'inner and outer rhizosphere', 'rhizocylinder', 'rhizoplane' etc. however, intrinsic heterogeneity of soil in the zone makes such categorization extremely difficult and often ambiguous (Sarkar and Jones,1980a).

The rhizosphere is often conceptualized as a small volume of soil clinging to short root segments, but the rhizosphere extends past the physical association of root and soil particles to a more complex volume of overlapping and functionally integrated zones. Within the rhizosphere, roots forage for soil-based resources, nutrients flux between organic and inorganic pools, mediated by the soil microbial community, and animals graze across trophic levels. The rhizosphere has major implications for climate and environment change with regards to greenhouse gas emissions and carbon sequestration, soil fertility management, and food security. The most succinct and clear definition of 'rhizosphere' is arguably the original definition of Hiltner (1904): 'soil influenced by roots'. Since that time, many developments have augmented the understanding of roots and the soil in which they live, and along the way different researchers in distinct disciplines have coined new words and changed definitions to suit their needs. Reviewing the broad literature on the rhizosphere, highlighting knowledge gaps, and identifying future research are necessary to advance our understanding of the interactions between roots and soil. Central to this consideration will be the adoption of systematic definitions and conceptual models that will allow greater synthesis of rhizosphere concepts and facilitate interdisciplinary collaboration (York et al, 2016).

Hence, the rhizosphere is subject to the influence of chemicals extracted by roots of living plants and the microbial community in this micro-zone. In the rhizosphere, competition between microorganisms and plants occurs for both iron and phosphorus demand, being microorganisms more competitive for their capacity to break down plant chlators and plants more able to counteract direct competition with microorganisms. Having large surface area, the active uptake of water and minerals through root hairs is highly efficient. Root hair cells secret acid (H^+ malic acid) which exchanges and helps solubilise the minerals into ionic forms, making ions easier to absorb. All processes and functions taking place in the rhizosphere are dominated by the activities of plant roots, rhizosphere micro-organisms interactions and enzymes are recognized as the main actors of all activities occurring in the rhizosphere environment. The production and activity of rhizosphere enzymes is controlled by several factors, in turn depending on soil-plant-microorganism interactions. In general, higher activity of rhizosphere enzymes can be interpreted as a general fuctional diversity of the microbial community. The lack of satisfying methodologies for accurately measuring the location of and activities of rhizosphere enzymes have often hampered clear knowledge and

understanding of their properties and functions. Sophisticated technologies now available will be helpful to reveal the origins, locations and activities of enzymes in the rhizosphere.

Organisms found in the rhizosphere include bacteria, fungi, comycetes, nematodes, protozoa, algae, viruses, archaea, arthropods etc. Rhizosphere organisms that are deleterious to plant growth and health include pathogenic fungi, comycetes, bacteria and nematodes-briefly termed as soil borne pathogens. Rhizosphere Effects (REe) are defined as biological, chemical, and physical changes in soils that occur because of root exudates and rhizo-deposition. Biological processes are related to the parasitism of host plant microorganisms that promote nutrient and water uptake from soils. The rhizosphere inhabiting microorganisms often compete for water, nutrients, space and sometimes improve their competitiveness by developing an intimate association with plants (Hartmann *et al,* 2009). These microorganisms play important roles in growth and ecological fitness of their host.

The Rhizosphere Effect and Rhizosphere Management Principles

During seed germination and seedling growth, the developing plant interacts with a range of microorganisms present in the surrounding soil. As seeds germinate and roots grow through the soil, the release of organic material provides the driving force for the development of active microbial populations in a zone that includes plant root and surrounding soil in a few mm of thickness. This phenomenon is referred as the rhizosphere effect (Morgan *et al.*, 2001). Broadly, there are three distinct components recognized in the rhizosphere; the rhizosphere *per se* (soil), the rhizoplane, and the root itself. The rhizosphere is thus the zone of soil influenced by roots through the release of substrates that affect microbial activity. The rhizoplane is the root surface, including the strongly adhering root particles. The root itself is a part of the system, because certain endophytic microorganisms are able to colonize inner root tissues (Bowenand Rovira*,* 1999). The rhizosphere effect can thus be viewed as the creation of a dynamic environment where microbes can develop and interact.

The rhizosphere (root-soil interface) is the most important area for plant-soil-microorganism interactions, and is the hub for controlling nutrient transformation and plant uptake (Zhang *et al.*, 2010, 2016, 2017), particularly for P due to its high fixation, low mobility, and low bioavailability in soil. Although the rhizosphere is often conceptually considered to be a thin layer of soil surrounding the root, the rhizosphere is actually a wider, interactive dynamic zone affected by various soil physical, chemical, and biological processes (York *et al.*, 2016). Plants are able to sense changes in their surrounding environment and optimize the absorption of water and nutrients by modifying rhizosphere processes.

Keeping an appropriate supply intensity of nutrients in the root zone can promote root growth and enhance rhizosphere processes, but a limited or oversupply of nutrients can repress these positive effects. Although plants have developed adaptive mechanisms to their environmental conditions through evolution, it is important that we maximize beneficial rhizosphere processes to take full advantage of the biological potential of roots to improve nutrient use efficiency and crop productivity in farming systems.

The rhizosphere is defined as the narrow region (1-2 mm) that surrounds the roots of land plants. Rhizosphere activity and processes are strongly influenced by plant exudates and soil microorganisms. Plants exude various primary and secondary metabolites through the rhizoplane to the neighboring soil rhizosphere that protect the root system from soil pathogens, attract and select useful microorganisms and interact both positively and negatively with other plants, thereby promoting plant growth. The interaction between the plant rhizosphere and surrounding soil is becoming a research hotspot for chemical ecologists. Researchers are now equipped with novel technologies including Next Generation Sequencing, the use of soil profiling and microprobes for genomics, transcriptomics and metabolomics studies, and sophisticated techniques for culture of novel organisms, all of which significantly accelerate our ability to conduct informative studies in soil systems, including those addressing chemical ecology in the rhizosphere, and the role of plants and microorganisms in these interactions.

Rhizosphere management strategies emphasize maximizing the efficiency of root and rhizosphere processes involved in nutrient mobilization, acquisition, and use by crops, rather than relying solely on the high use of mineral fertilizers in intensive farming systems (Shen *et al.*, 2013; Jiao *et al.*, 2016). Rhizosphere management strategies include: 1) regulating root morphology and architecture by adjusting the quantity, composition and manner of nutrient supply; 2) increasing the bioavailability of sparingly soluble nutrients by manipulating root exudation; 3) improving the uptake of immobile nutrients by employing mycorrhizal fungi and other beneficial microorganisms; 4) intensifying rhizosphere interactions through interspecies interactions by intercropping (Figure 1). The overall goal of rhizosphere management is to increase nutrient use efficiency, improve crop yields, optimize mineral fertilizer inputs, and achieve sustainable crop production by optimizing and integrating a range of beneficial rhizosphere interactions.
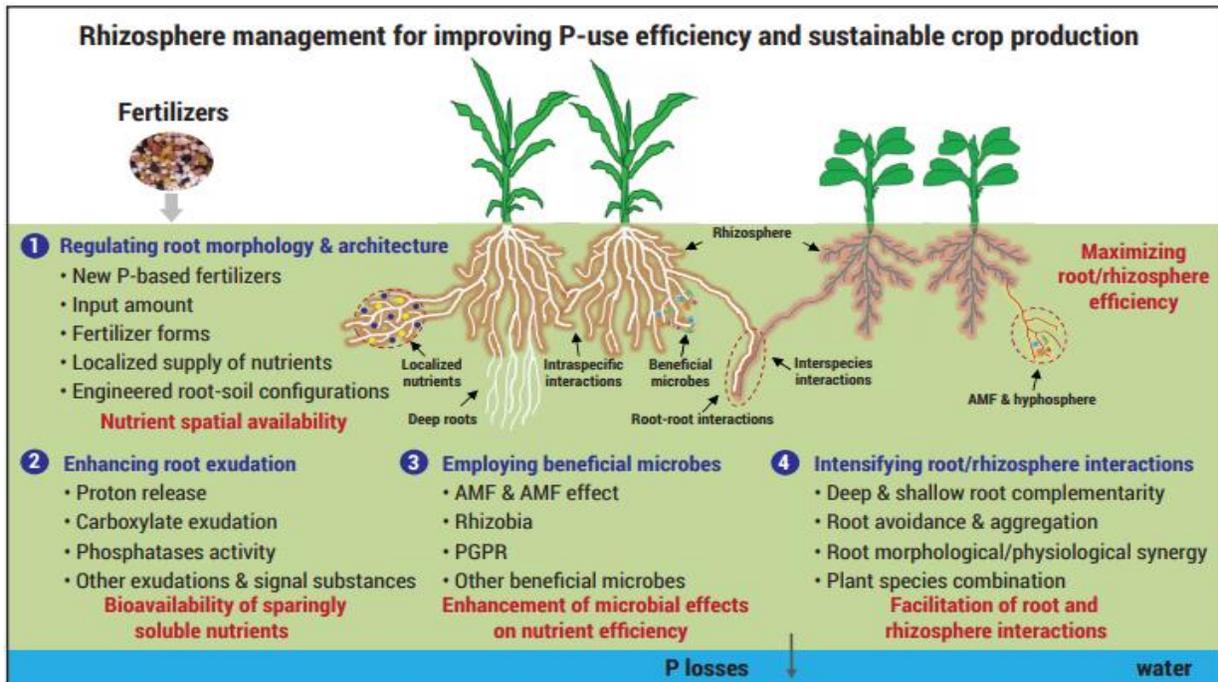

Figure 1: Rhizosphere Management Strategies for Sustainable P use in cropping systems

Micromorphology of Rhizosphere: A Literature Review

Micro-morphological investigations have been used in wide range of studies for both characterization and classification of fabric (Brewer, 1964; Stoops & Jongerius, 1975) including that of humic microfabric (Barrat, 1969; Babel, 1965, 1975) and of specific features such as oriented clay cutans, nodules and voids. Specific micro-morphological techniques namely transmission (Jenny and Grossenbacher, 1963) and scanning electron microscopy (Dart, 1971; Campbell

Rovira, 1973) have also been used to illustrate the special relationship of soil-root-microorganisms in undisturbed rhizosphere. The physical and nutritional environment is highly dependent on the very nature of the fabric around the root (Barley, 1954, Sedgely and Barley, 1958; Halstead *et al*, 1968). Blevins *et al* (1970) gave a detailed physical description of fabric at the tree-root-soil interface.

Micro-morphology is the branch of soil science that is concerned with the description, interpretation and to a certain extent, the measurement of soil components, unique features and fabrics (i.e. micro-pedons) in soils at microscopic level (Bullock *et al,* 1985). The first thin section studies of soils date from the beginning of the 20th century. Since then, soil micromorphology has gradually gained importance in research, several systems of thin section description were developed, each using specific concepts and terminology, and new methods were applied. In the beginning its main use was in the field of soil genesis, as well as soil classification, although its practical application in determining soil types is hindered by many factors inherent to the present classification systems. Micromorphology is a precious tool in palaeopedogy to disentangle polygenetic processes pointing to changes in climatic/environmental conditions over time. For the same reason it became an important tool in geo-archaeological research. There is a need for more extensive correlation with physical and chemical data, especially as a contribution to pedometric studies and soil management. It can also be a great help in monitoring field and laboratory experiments ((https://doi.org/10.1016/B978-0-444-63522-8.00001-2).

On a similar line, Scarciga *et al* (2009) used an innovative methodological approach based on the chemical analysis of different portion of soil horizons (alluvial pedofeatures, pedogenic matrix and skeletal parent rock fragments) by laser ablation inductively coupled plasma mass spectroscopy (LA-ICP-MS) associated with traditional micro morphological techniques such as: optical and scanning electron microscopy. Validation of LA-ICP-MS techniques provided *in situ* accurate and reproducible RSD: 13-18% analysis of low concentration trace elements and rare earth elements (REE) in soil samples of 0.001-0.1 p.p.m. concentrations.

Microscopic bodes of various forms, dimensions and morphology have been observed in the soil suspension under electron microscope (Nitin, 1964). Some of the limitations of direct electron microscopy have been minimized by the introduction of 'Scanning electron Microscopy' (S.E.M.). The S.E.M. has several advantages including those of minimal preparation, considerable depth of focus, high magnification, and high resolution with three dimensional effects. The technique has been used with varying degrees of success, by several workers (Marchant, 1970; dart, 1971; Campbell and Rovira, 1973; Rovira and Campbell, 1974, Sarkar and Wyn Jones, 1980b, Sarkar and Jenkins, 1980) to observe microbial association of the root surface and their distribution in the rhizosphere. Experimentally, the rhizosphere has been sampled in various ways that have led to different functional definitions being used in soil science, microbial ecology, and plant biology.

The authors have outlined the problems with 'rhizosemantics' above and encourage researchers to be more consistent with their terminology by referring to the root surface as the root epidermis; the adhering soil and binding materials, such as mucigel, as the rhizosheath; and the combination of the epidermis and rhizosheath as the rhizoplane (Figure 2), which is one component of the holistic rhizosphere in agreement with Puente *et al.* (2004). This synthesis of the terms allows a new exploration of a holistic rhizosphere composed of overlapping and integrated zones. The rhizosphere is holistic because the structure and function of rhizosphere components can only be understood by reference to the entire rhizosphere construct and the relations between components.

Figure 2: A cross section of Barley roots showing rhizosheath, rhizoplane and rhizosphere
Source: Micrograph provided by Margaret McCully from Hinsinger *et al.* (2003)

A barley root sampled from the field is depicted with its rhizosheath, soil particles bound by root hairs, and mucigel. The rhizoplane includes both the root epidermis and the rhizosheath, while the rhizosphere may extend beyond the boundaries of the rhizosheath. Micrograph provided by Margaret McCully from Hinsinger *et al.* (2003).

Roots also affect the physical structure of the soil by creating a zone of soil structure modification (SSM). As the growing tip of a root burrows through soil, particles are displaced that can form a zone of higher density soil around roots. The SSM zone concept was supported by earlier work investigating soil deformations using radially expanding tubes (Dexter and Tanner, 1972), and by subsequent measurements around roots grown in field soil (Bruand *et al.*, 1996). Braunack and Freebairn (1988) found a reduction in porosity immediately adjacent to the root using radiographic methods, which they argued, was due to soil compression as the root expanded. Aravena *et al.* (2011, 2014) showed root-induced soil compaction can increase root-soil contact, which has key implications for hydrological behaviour in this zone that they demonstrated using modelling approaches. Thus, soil porosity is generally believed to decrease at the root-soil interface. However, other research showed a general increase in porosity in the presence of roots even over timescales of a few weeks (Feeney *et al.*, 2006). Most studies have used different species and soil types, so the generality of how roots affect soil structure is not known. Beyond this SSM zone immediately at the root-soil interface, roots and root exudates stabilize soil aggregates at several spatial scales (Tisdall and Oades, 1982).

Soil Chemistry and Plant Nutrition of Rhizosphere: a Literature Review

Plant Rhizosphere is the soil nearest to the plant root system where roots release large quantity of metabolites from living root hairs or fibrous root systems. These metabolites act as chemical signals for motile bacteria to move to the root surface but also represent the main nutrient sources available to support growth and persistence in the rhizosphere. Some of the microbes that inhabit this area are bacteria that are able to colonize very efficiently the roots or the rhizosphere soil of crop plants. These bacteria are referred to as plant growth promoting rhizobacteria (PGPR). They fulfil important functions for plant growth and health by various manners. Direct plant growth promotion may result either from improved nutrient acquisition and/or from hormonal stimulation. Diverse mechanisms are involved in the suppression of plant pathogens, which is often indirectly connected with plant growth (Nihorimbere *et al,* 2011).

The works of Barber (1962), Nye and Tinker (1969) have emphasized the importance of the movement of nutrients, either by mass-flow of diffusion process to the

root surface. The various experimental observations generally indicate that the major portion of Ca and mg reach root surface by mass-flow, whereas diffusion is the mechanism for supplying plants with K and P (Barber *et al,* 1963). Root interception and mass-flow are the significant mechanisms for uptake and supply of Fe, B, Cu and Sr to plant roots (Oliver and Barber, 1966).

Specific effects of various fertilizer applications on the availability of nutrients have not received adequate attention so far as it rightly deserves. All the same, it has been well established that the application of nitrogen from various sources benefits the availability of macronutrients and micronutrients in soil, either by way of increase of decrease in their availability to growing plants, depending on the ionic forms present in fertilizer sources (Blair et al, 1970, miller et al, 1970, Riley and Barber, 1969; Sarkar and Wyn Jones, 1980a). A pH drop in the root environment applying ammonia (Sarkar and Wyn Jones, 1980b) and choline and a pH increase following application of nitrate (Sarkarkar and Wyn jones, 1980b) have been observed in classical studies. The Rhizosphere effect of ph on the availability of various nutrients in soil is widely known (Denahue, 1965). Effects of plant-microbe interactions (Rhizosphere effect) on nutrients uptake have also been reviewed (Sarkar and Wyn Jones, 1980b).

A number of workers have shown that that the availability of micronutrients to plants depends to a large extent on the soil pH (Loneragan, 1975; lutz et al, 1972; Olomu et al, 1973). Interdependence with soil has also been observed (Mukhopadhya et al, 1967; Olomu et al, 1973; Patrick and Dalaune, 1972). The manipulation of rhizosphere pH by using NH_4^+ - N and NO_3-N was pioneered by Riley and Barber (1971) and relatively recently by Sarkar and Wyn Jones (1982) have elaborated on this method by using choline as well as ammonia salts to produce low rhizosphere pHs. In this and other studies, including studies relating to Micro-morphology of rhizosphere; Sarkar et al, (1976, 1979); Sarkar and Jenkins, (1980), Sarkar and Wyn Jones (1981) have made a significant contribution on Micromorphology of the Rhizosphere with special reference to plant nutrition. The paper highlights some of their findings along with other workers working on similar lines.

Micromorphology of the Rhizosphere:

Methodology and Findings

Methodology for Part I of the Study

Sarkar *et al* (1976), Sarkar and Jenkins (1979) and Sarkar *et al* (198a & 1980b) are some of the early among the research workers to conduct research studies, among other themes, on the "Micromorphology and Biochemistry of Soil Science (Sarkar, 1977) on dwarf French b eans grown in controlled greenhouse

conditions. Since then, soil micro-morphology with specific focus on the rhizosphere has gradually gained importance, particularly using thin sections with optical polish < 1 micron, drawn out of rhizosphere soil core embedded into epoxy resin material.

Refinement of this technique has been tried and tested with varying degree of demonstrative success in different research laboratories in the world; each of course using different concepts, approaches, objectives and terminologies. For instance, in the beginning, its main use was in the field of soil genesis, as well as soil survey and classification. Micro-morphology is a precious tool in paleopedology to disentangle polygenetic processes pointing to changes in climatic/environmental conditions over a certain period of time horizon. And precisely for the same reason, over a period of time, it became an important tool in geo-archological research investigations.

Reverting to the original work of Sarkar *et al* (1976) on "Modifications to Mechanical and Mineralogical composition of soil within Rhizosphere' it was found during factorial experiment study of rhizosphere of rhizosphere development around French bean roots, the mechanical and mineralogical modifications were found obvious and recorded. Whereas, the former entailed in relative decrease of material ranging from 63-6.3 Micro-m e.s.d. in size; probably due to disaggregation of polymineralic shale particles, the latter was more obvious in the finest (i.e. <0.063micro m.) fraction and involved a decrease of both regularly and irregularly interstratified 10/14 Angstrom material such as; "amorphous" (extractable) Al, Fe and C accumulated in the rhizosphere; whilst Si was depleted. These effects were interpreted as being due to "weathering "of soil materials in the vicinity of the plant root- i.e. Rhizoplane.
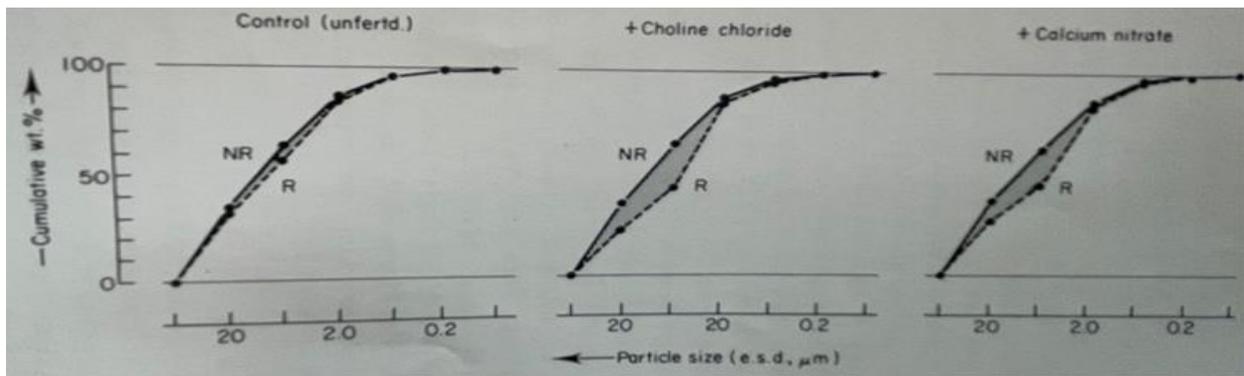

Figure 3:   Mechanical analysis of Rhizosphere and Non-Rhizosphere soil samples

Sarkar *et al* (1979) had pursued their study a step further by conducting research on "Detection of Elemental concentration change in the Root environment by employing Contact Micro-Autoradiography Technique". This particular technique involved contact exposure with irradiated (^{45}Ca and ^{32}P).

Thin sections prepared from impregnated soil blocks, and subsequent development and fixing of the films were carried out. Relative effectiveness of the detection of elemental concentration changes of Ca and P in the vicinity of roots were tested using X-ray films (i.e. industrex fine grade film and Kodak stripping film).
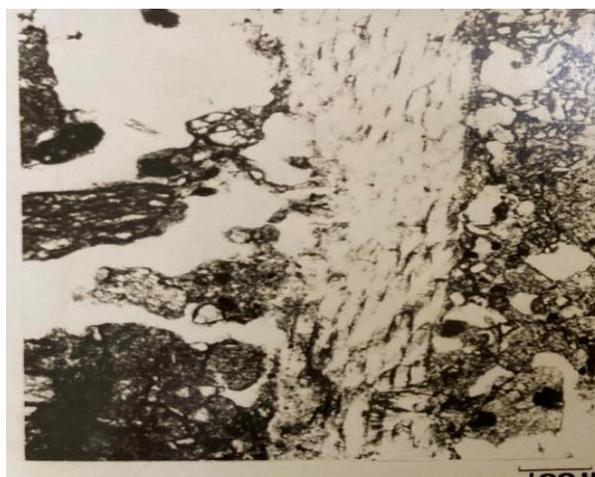

Figure 4: Rhizosphere Photograph

Figure 5: Rhizosphere Autoradiograph (^{45}Ca)
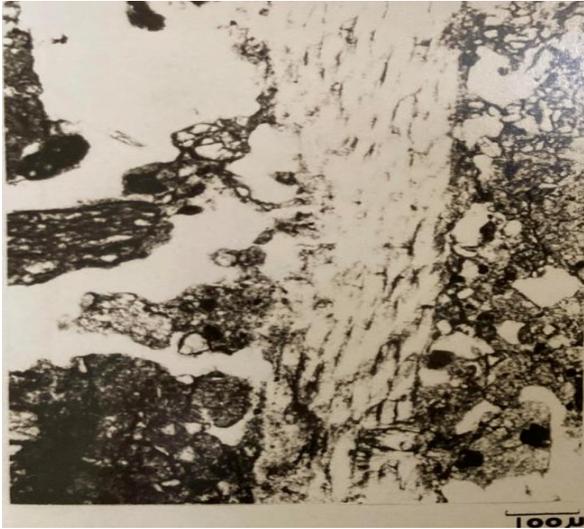
Figure 6: Rhizosphere Photograph   Figure 7:   Rhizosphere Autoradiograph (^{32}P)

Research Findings of Part I of the Study

Of the two, stripping film was found to be most suitable. Thus, using this film, gradient in concentration of Ca in the vicinity of roots was detected in case of dwarf French beans receiving choline phosphate treatment. However, when dwarf French bean received calcium nitrate treatment, Ca was shown to have accumulated on the soil-root interface i.e. rhizoplane. No depletion zone of P was detected as such with either of the fertilizer treatments. However, in case of Choline phosphate treatment P was found to have migrated to endodermal layer (i.e. endodermis). General association of P with organic debris (mostly humic substances) of root or microbial origin or even a mix of the two as well as sesquioxidic materials was also visibly apparent.

A further follow-up of the above mentioned study was subsequently carried out by Sarkar and Jenkins (1979) titled "Micromorphology of the Rhizosphere' revealed that thin sections (<25 micron) prepared from the resin impregnated blocks- both of the rhizosphere and control soil were examined under microscope. The fabric of control soil was found to be characterized by the presence of large grain and irregular 'orthovughs', sporadically distributed in the fabric with high grain/plasma ratio. The S-matrix of the rhizosphere fabric was described as integrated "Humicol-Agricol." Insepic plasma fabric extending upto 250 micron from the rhizosphere was detected. No preferential orientation of the fine grains were noticed on the root surface, although very fine fabric components of organic and inorganic sources intermixed with root hairs (fluoresced brown), adhered with partially decayed cortical cells forming "micro-aggregates"/micropedons or simply "clusters". This special fabric feature was described as "spongy mullicol".

Methodology and Findings of Part II of the Study

Sarkar and Jenkins (1980a) conducted further studies on the theme of micro-morphology of the rhizosphere titled "Detection of Elemental concentration change in the root environment using Electron-Probe X-Ray Analysis (EPRA) Technique" (Refer Fig. 7, 8, 9, 10). In this study, a representative thin section prepared from the undisturbed and homogenized block of rhizosphere soil, receiving choline phosphate treatment and subjected to micro-probe x-ray analysis. Three selected sites in the vicinity of roots were studied. Maintaining the sample field stationary, the electron beam was allowed to scan along a selected line across rhizospheric fabric perpendicular to root. Independent scanning profiles relative to concentrations of P, Ca, Fe, Mn, Al and Si were presented superimposed. Similarly, between the scanning profiles of Ca and P in selected 2-3 fields were studied; which suggested a possible mutual association of these elements in the root interior as well as in the rhizosphere. A relatively high concentration of P in the root and on the rhizoplane compared to the neighbouring matrix suggested a possible depletion of this element (i.e. P) in the vicinity of root i.e. rhizoplane. Trace elements like Fe, Al and Mn tended to concentrate remotely from the root surface and did not show association with phosphate in the root environment.
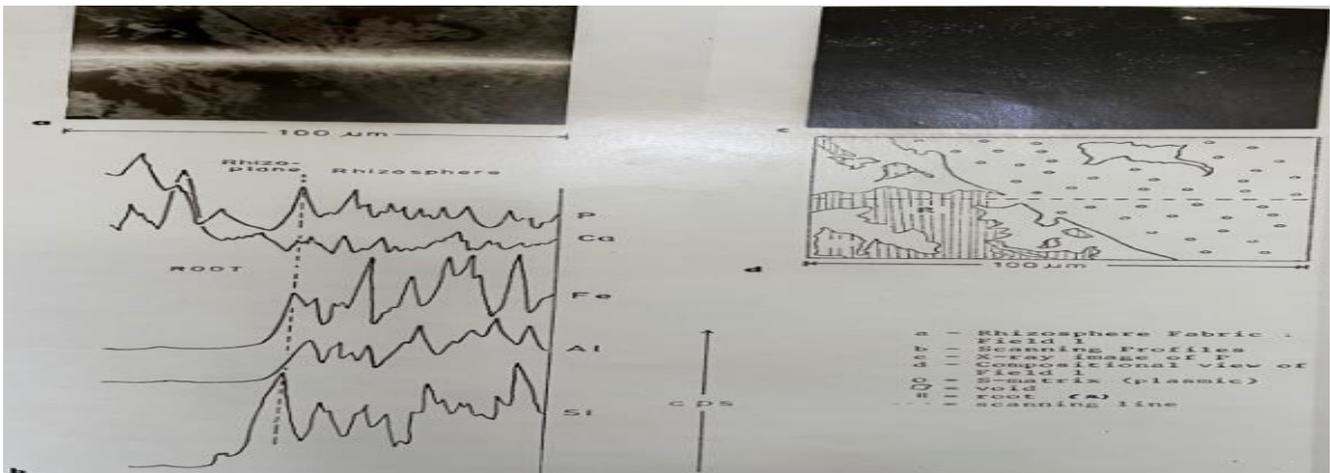
Figure 8: Electron-Probe X-Ray Analysis of Rhizosphere Fabric - Field 1 of study
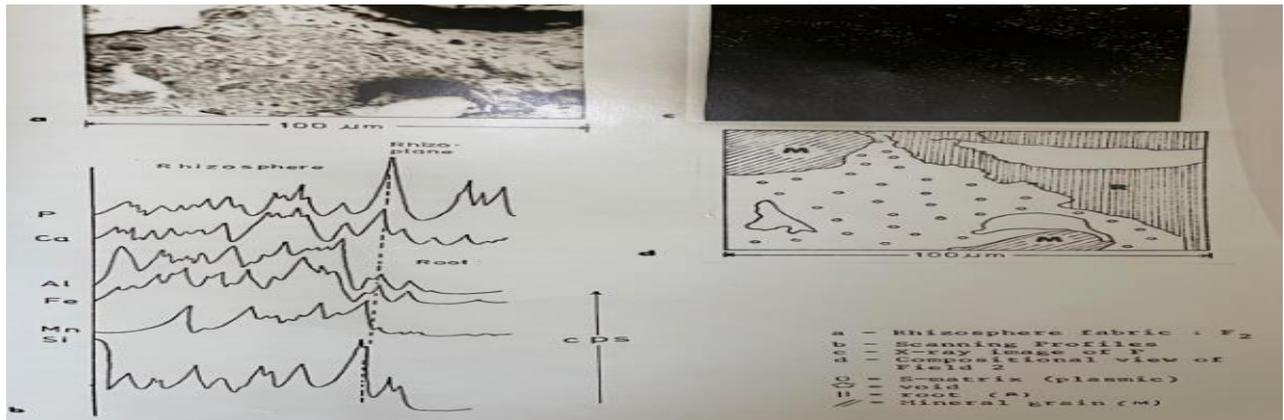

Figure 9: Electron Probe X-Ray Analysis of Rhizosphere Matrix- Scanning profile, X-Ray image and Composite view- Site 2
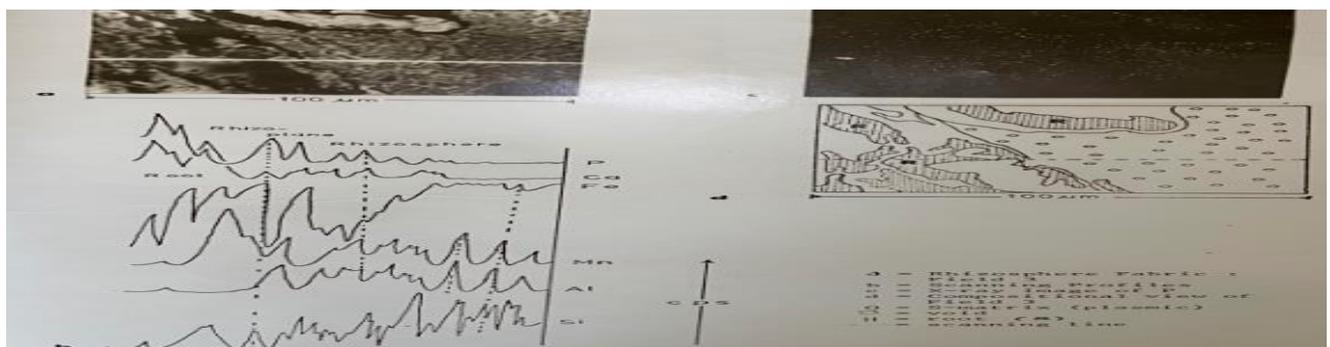

Figure 10: Electron-Probe X-Ray analysis of Rhizosphere Fabric in Field 3

As a follow-up of the earlier study by Sarkar and Jenkins (1980), Sarkar and Wyn Jones (1982a) conducted a study on "Effect of Rhizosohere pH on the availability and uptake of Fe, Mn and Zn" (Figure 11). In this study, a number of pot experiments on finding the relationship between Rhizosphere pH, the extractable levels of Fe, Mn and Zn in the soil and their uptake into the roots and shoots of dwarf French beans were studied. Variations in the rhizosphere pH were induced by applying three different sources of nitrogen- choline phosphate, ammonium phosphate and calcium nitrate to an initially homogenized soil (pre-adjusted to either pH 7 or pH 8). The rhizosphere ph was found to be significantly lower following the application of either

ammonium or choline phosphates and to be increased by calcium nitrate treatment (Figure 12). The Fe and Zn contents of both shoot and root were found to be inversely proportional to Rhizosphere pH (Figure 13). The Mn contents also increased with increasing pH; but a sharp increase was apparent below pH 5.5. The shoot Fe, Mn and Zn contents were significantly correlated with the extractable levels determined in the Rhizosphere and non-Rhizosphere (control) soil (Figure 14 and Figure 15).
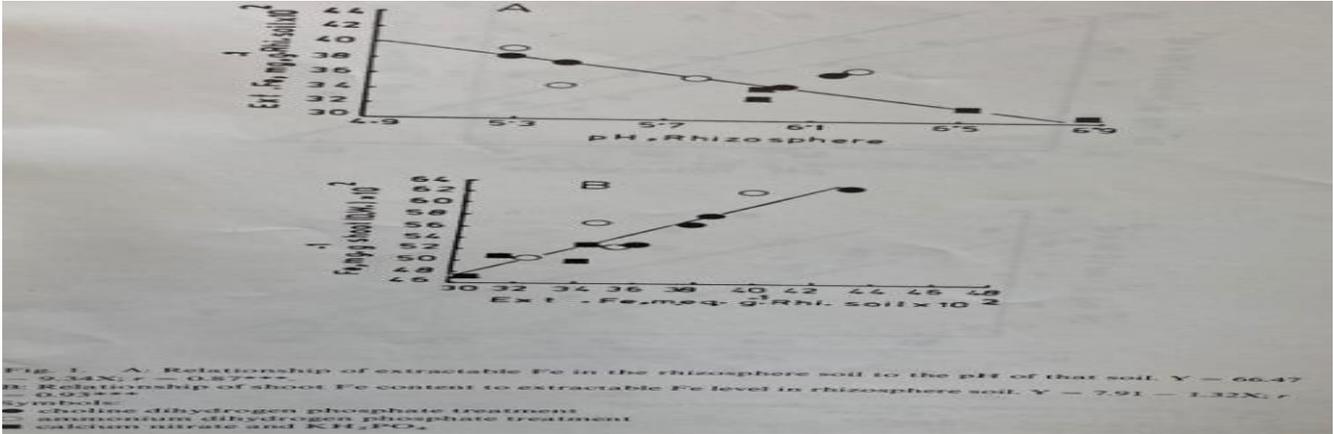

Figure 11: Effect of Rhizosphere pH on uptake of Fe, Zn and Mn
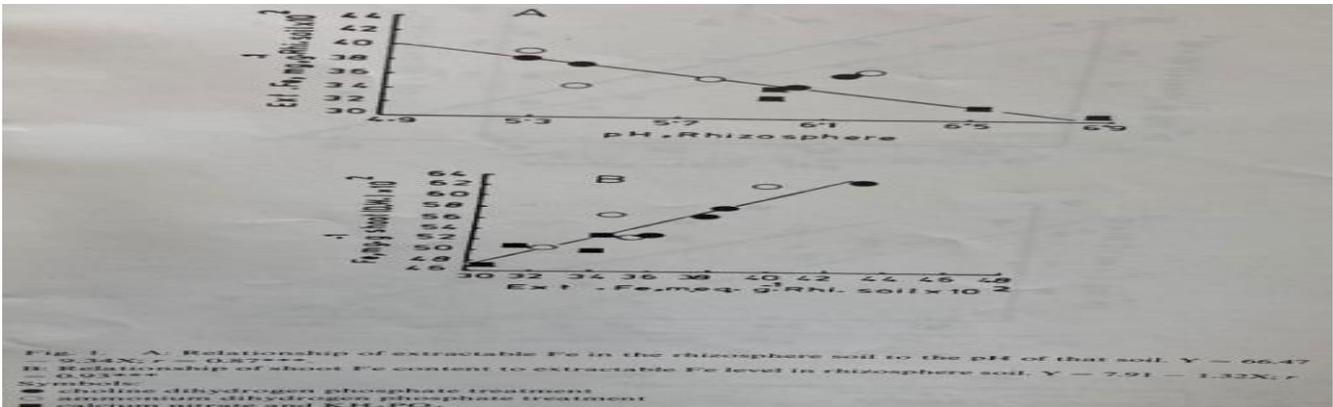

Figure 12: Relationship between Fe content in shoot and pH level of Rhizosphere soil
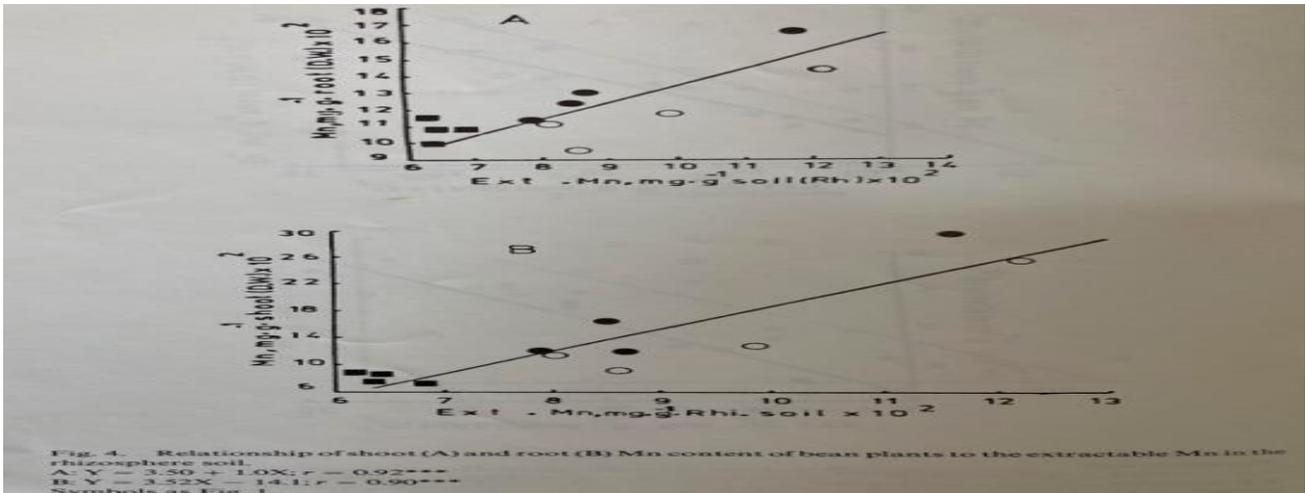Figure 13: Relationship between Fe content in shoot and extractable Fe level of Rhizosphere soil

Figure 14: Relationship of shoot (A) and root (B) Mn contents in French beans plants to the extractable Rhizosphre soil Mn content

Fig. 3.   Relationship of shoot (A) and root (B) Zn content of bean plants to the extractable Zn in the rhizosphere soil.
A: Y — 0.452X — 0.57; r — 0.959***
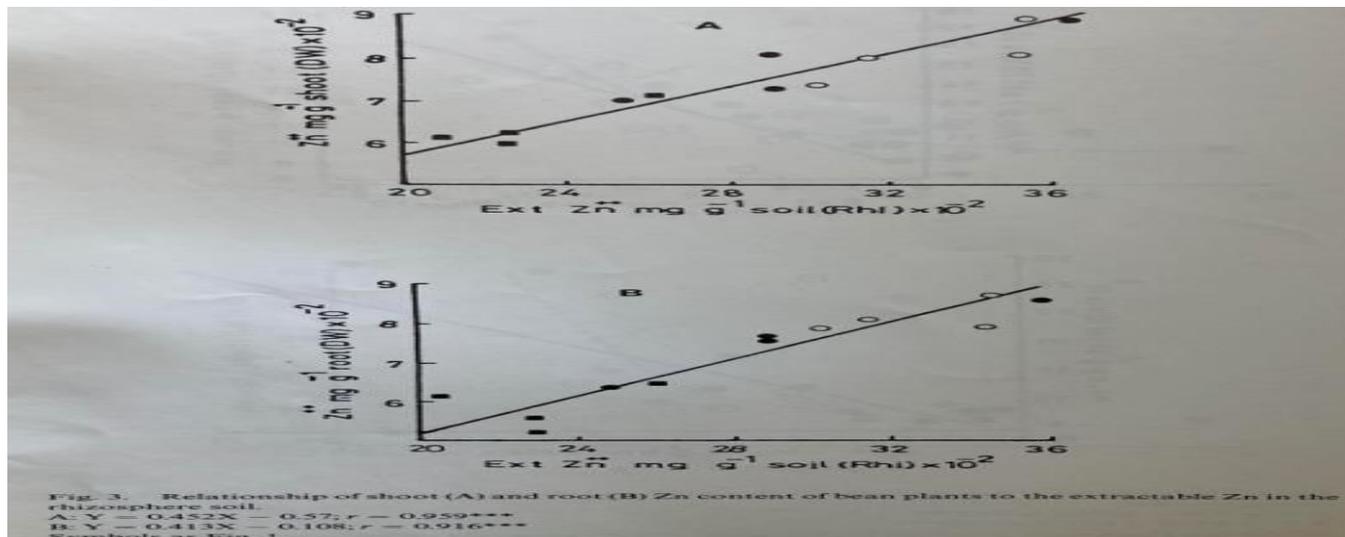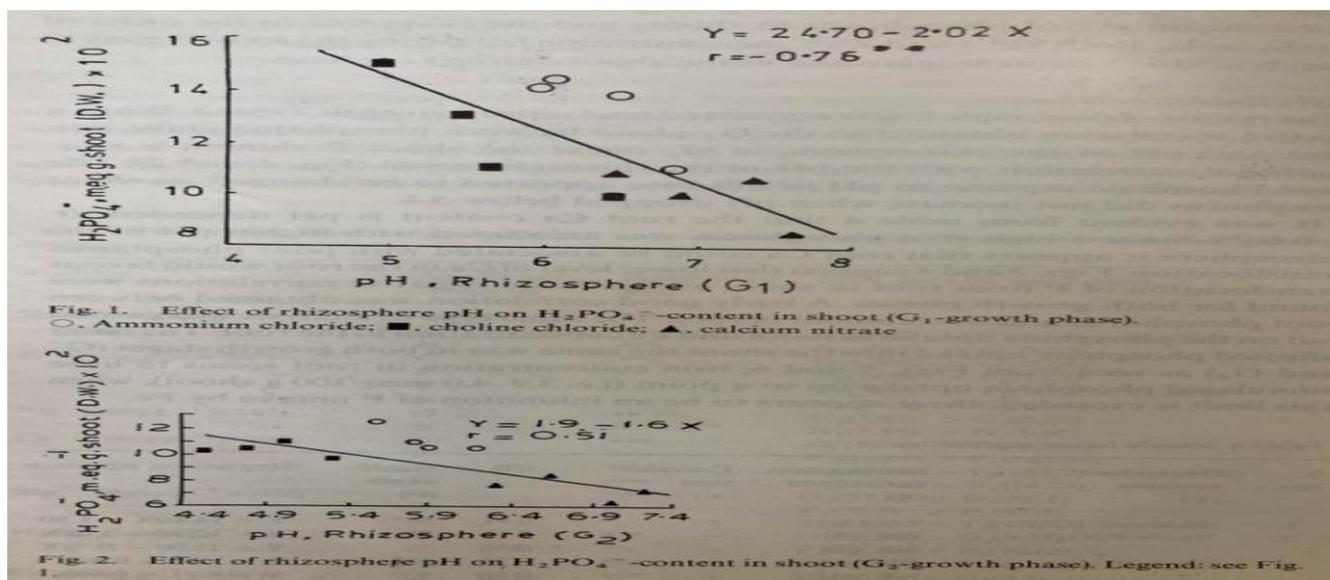B: Y — 0.413X — 0.108; r — 0.916***
Symbols as Fig. 1.

Figure 15: Relationship between shoot (A) and root (B) Zn contents in French bean plants to the extractable Zn in Rhizosphere soil

Another study by Sarkar and Wyn Jones (1982b) followed soon after the previous study. This study was on "Influence of Rhizosphere on the nutrients status of dwarf French beans". In this study, French bean seedlings were grown on choline, ammoniacal and nitrate forms of nitrogenous fertilizers, together with equivalent basal applications of P as KH_2PO_4 were tested for nutrients uptake from the Rhizosphere soil. Statistical tests on soil (both Rhizosphere and non-Rhizosphere0 and plant (root and shoot) revealed that with the exception of P, levels of all other estimated macro – (Na^+, K^+ Ca^{2+} , Mg^{2+}) were significantly changed after 42 days plant growth as compared to 21 days growth period in the pot experiment (Figure 16). The higher uptake into shoots of Na^+, K^+, Fe^{2+} Mn^{2+}, Zn^{2+} and $H_2PO_4^-$ and higher biomass accumulations in the Rhizosphere were associated with lower Rhizosphere pH (Figure 16).

Figure 16: Effects of Phosphatic and Ammoniacal fertilizer applications on pH and H_2PO_4 contents of shoot at Growth Stage 1 and Growth Stage 2

Fig. 1.   Effect of rhizosphere pH on $H_2PO_4^-$-content in shoot (G_1-growth phase).
○, Ammonium chloride; ■, choline chloride; ▲, calcium nitrate

Fig. 2.   Effect of rhizosphere pH on $H_2PO_4^-$-content in shoot (G_2-growth phase). Legend: see Fig. 1.

The uptake of Ca^{2+} and Mg^{2+} increased with higher Rhizosphere pH. Whilst, ammoniacal and choline forms of fertilizer applications decreased Rhizosphere pH and increased P uptake; nitrate form on the contrary reversed the trend showing significant inverse relationship between shoot phosphate and Rhizosphere pH. Ca and Fe were associated with an inhibition of translocation of P form from root to shoot. However no causal relationships could be established in such relationship. Both shoot weight and shoot phosphate

contents were closely associated with a number of Rhizosphere soil parameters and much less so with the non-Rhizosphere soil parameters.

Rhizosphere Models and Computer Simulations

Rhizosphere modelling is not common, and has focused mostly at millimetre scales with little upscaling. In contrast, modelling of root systems with water and nutrient uptake has advanced significantly (six such models are reviewed in Dunbabin *et al.*, 2013), yet soil is typically modelled entirely as bulk soil with no influence of the roots on soil properties. However, rhizosphere models can be informative, and likely have profound impacts on larger scale systems. For example, a rhizosphere model of a growing root demonstrated stable changes in soil pH occurring within 6h with a 1mm accumulation zone, and that measurements using agar overestimated the size of the accumulation zone due to increased diffusion (Kim *et al.*, 1999). A single root simulation of exuded mucilage and water uptake demonstrated greater benefits at greater water uptake rate potential and when mucilage didn't diffuse as far (Ghezzehei and Albalasmeh, 2015). Another model of water uptake extended the Tardieu-Davies model to include circadian rhythms of stomatal and root hydraulic conductance based on the rhythm of ABA concentrations, and this model could be combined with both genetic regulatory models and whole plant or population models (Tardieu *et al.*, 2015). Clearly, considering the rhizosphere is necessary in root structural-functional simulations.More robust soil models including the dynamics of microorganisms will be especially important in future research of the rhizosphere.

CONCLUSIONS

The rhizosphere has been defined in terms of the effects of roots on soil microorganisms (Toal *et al.*, 2000), the depletion of water (Segal *et al.*, 2008), changes in pH (Kim *et al.*, 1999), adhering soil (Bulgarelli *et al.*, 2012), and so on. Hiltner (1904) defined the rhizosphere as the soil influenced by roots, so though reductionist research led to more narrow conceptions and to a greater understanding of individual processes, the interdisciplinary research of the future must acknowledge a dynamic region of interacting processes: the holistic rhizosphere. However, in acknowledging the rhizosphere as a 'whole in reciprocal interaction with its own parts' (Levins and Lewontin, 1980), that the rhizosphere itself is but a part of a greater soil system must also be realized. By using integrative methods including non-destructive imaging, next-generation chemical assays with substantial spatiotemporal resolution, and simulation modelling, the secrets of the dynamic rhizosphere will be revealed. Holistic rhizosphere science has the potential to substantially increase understanding of plant-soil systems and provide guidance for pressing issues of the 21st century, such as agricultural sustainability and environmental change.

A systematic review of research investigations and pioneering work done by Sarkar and associates in Bangor, U.K.; Stanley A. Barber and associates in Purdue, U.S.A., Nye and Tinker and associates in Oxford, U.K.; as well as other research workers in different parts of the world in the 20 th Century, mainly relating to micro-morphological investigations needs special recognition as it deserves. These experiments have often been combined with parallel pot and/or field experiments; and closely corroborated with analysis of plant and shoot samples (tissues) as are derived from test crop(s) at different stages/period of growth. These experiments have given interesting; and to a great extent, conclusive results: the details of which are given in the paper, which are self-explanatory. It would appear from the ongoing trends of research studies in different laboratories that there is a clear need to evolve, develop, try, test and adapt some of the some of the recently developed and advanced techniques such as the use digital sensors, IoT techniques, Artificial Intelligence (AI) techniques etc. to help increase the proper sampling methodology (ies) of drawing Rhizosphere samples for testing, enhancing depth of focus, higher resolution for micro-morphological investigation, relying analytical and statistical data which are drawn from *in situ* samples rather than *ex-situ* samples. Electrochemical Sensors now-a-days being widely used under AI can help provide key information/data required for precision agriculture: pH and soil nutrients levels in particular. Sensors electrodes work by detecting specific ions in soil, Currently, sensors mounted to specifically designed "sleds" help gather, process, map soil chemical data. Such AI techniques will have accuracy, precision and reliability over and above the current techniques of nutrients movement, concentration, depletion and competition scenario analysis with fair degree reproducibility and validity.

A better understanding of the basic principles of the rhizosphere ecology, including the function and diversity of inhabiting microorganisms is on the way but further knowledge is necessary to optimize soil microbial technology to the benefit of plant-growth and health in the natural environment. In sum, this can constitute overwhelming evidence indicating that an ever exploitation of plant growth promoting rhizobateria (PGPR) can be a true success story in sustainable agriculture.

As a consequence, current production methods in agriculture, e.g., the improper use of chemical pesticides and fertilizers creating a long list of environmental and health problems, will reduce.

REFERENCES

Aravena JE, Berli M, Ghezzehei TA, Tyler SW (2011), Effects of root-induced compaction on rhizosphere hydraulic properties – X-ray microtomography imaging and numerical simulations, Environmental Science and Technology, 45, 425–431.

Aravena JE, Berli M, Ruiz S, Suárez F, Ghezzehei TA, Tyler SW (2014), Quantifying coupled deformation and water flow in the rhizosphere using X-ray microtomography and numerical simulations, Plant and Soil, 376-110

Babel U (1965), Z. P flErnahr, Dung, 109, 17.

Babel U (1975), Soil components: Organic components (Ed. Giesseking, J.E.) Springer-Verlag, N.Y.

Barber SA, Ozanne PG (1970), Autoradiographic evidence for the differential effect of four plant species in altering the calcium content of the rhizosphere soil, Soil Science Society of America Journal, 34, 635 – 637.

Barber SA (1962), A diffusion and mass-flow concept of soil nutrient availability, Soil Sci.93, 39-49.

Barber SA (1962), A diffusion and mass-flow concept of soil nutrient availability, Soil Sci.93, 39-49.

Barber SA, Walker JW, Vassey EH (1963), Mechanisms for movement of plant nutrients from the soil and fertilizers to plant roots. J. Agr. Food Chem. 11, 204-207.

Barber SA, Walker JW, Vassey EH (1963), Mechanisms for movement of plant nutrients from the soil and fertilizers to plant roots. J. Agr. Food Chem. 11, 204-207.

Barrat BC (1969), Micromorphological aspects of arctic-alpine pedogenesis in the Okstindan Mountains, Norway, Geoderma, 2, 257.

Blair GJ, Miller MH, Mitchell WA (1970), Nitrate and ammonium as sources of nitrogen for corn and their influence on uptake of other ions. Agron. J. 62, 530-532.

Blair, G.J, Miller, M.H. and Mitchell, W. A. (1970), Nitrate and Ammonium as sources of nitrogen for corn and their influence on uptake of other ions, Agron. J. 62, 530-532.

Bowen G. & Rovira A. (1999). The rhizosphere and its management to improve plant growth. Adv. Agron., 66, 1-102.

Braunack,M. Freebairn, D. (1988),The effect of bulk density on root growth, Proceedings of the 11th International Conference of the International Soil Tillage Research Organisation, Edinburgh, 25- 30

Brewer, R. (1964), Fabric and Mineral Analysis of Soil, John Willey & Sons Inc. N.Y.

Bruand A, Cousin Nicoullaud B, Duval, O, Bégon, JC (1986), Backscattered electron scaning images of soil porosity for analyzing soil compaction around roots, Soil Science Society of America Journal, 60, 895–901.

Campbell, Rovira AD (1973), Soil Bioll. Biochem, 5, 247-252.

Dart, P.J. (1971), Expt. Bot. 22, 164-168.

Dexter AR, Tanner DW (1972), Soil deformations induced by a moving cutting blade, an expanding tube and a penetrating sphere, Journal of Agricultural Engineering Research, 17, 371–375.

Dunbabin, VM, McDermott S, Bengough AG (2006), Upscaling from rhizosphere to whole root system: modelling the effects of phospholipid surfactants on water and nutrient uptake, Plant and Soil, 283-57-72.

Feeney DS, Crawford JW. Daniell T, Hallett PD, Nunan N. Ritz K, Rivers M, Young IM (2006), Three-dimensional microorganization of the soil-root-microbe system, Microbial Ecology, 52, 151–158.

Ghezzehei, TA. Albalasmeh, AA (2015), Spatial distribution of rhizodeposits provides built-in water potential gradient in the rhizosphere, Ecological Modelling, 298, 53–63.

Hartmann et al, (2009), Beneficial effect of the Rhizosphere microbial community

Hiltner, L. (1904) Arb. Deut. Lander. Ges. 98, 59-78(ONS).

Hinsinge, P, Plassard, C, Tang, C, Jaillard, B (2003), Origins of root-mediated pH changes in the rhizosphere and their responses to environmental constraints: a review, Plant and Soil, 248, 43–59.

Jackson, M.L. (1964), Soil Chemical Analysis, Prentice Hall Inc.

Jenny H, Grossenbacher K (1963), Behavior of Ions in Soils and Plant Responses

Journal of Experimental Botany, Volume 67, Issue 12, June 2016, Pages 3629–3643, https://doi.org/10.1093/jxb/erw108

Kim TK, Silk WK, Cheer AY (1999), A mathematical model for pH patterns in the rhizospheres of growth zones, Plant, Cell & Environment, 22, 1527-–1538

Larry M York, Andrea Carminati, Sacha J. Mooney, Karl Ritz, Malcolm J. Bennett (2016), The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots

Levins R Lewontin, R. (1980), Dialectics and reductionism in ecology Synthese, 43, 47-–78

Marchant, R (1970), The root surface of Ammophila arenaria as a substrate for microorganisms. Trans. Br. mycol. Soc., 54 (1970), pp. 479-482.

Mehra OP, Jackson ML (1960), Iron Oxide removal from soil and clays by dithionate-citrate system with sodium bi-carbonate buffers, Clay Minerals Min 5, 317-668.

Morgan J.A.W. & Whipps J.M. (2001), Methodological approaches to the study of rhizosphere carbon flow and microbial population dynamics. In: Pinton A., Varanini Z. & Nannipieri P., eds. The rhizosphere. Biochemistry and organic substances at the soil-plant interface. New York, USA: Marcel Dekker, 373- 409.

Mukhopadhay A, Fisher TR, Smith GJ (1967), submergence and limiting effects of soil changes in pH, Eh and Mn uptake by rice plants. Soil sci. 104, 107-112.

Mukhopadhay A, Fisher TR, Smith GJ (1967), submergence and limiting effects of soil changes in pH, Eh and Mn uptake by rice plants. Soil sci. 104, 107-112.

Nitin DI (1964) Soviet Soil Science 6, 636-642.

Olomu MO, Raca GJ, Cho CM (1973), Effect of flooding on Eh, ph and concentration of Fe and Mn in several Manitoba soils. Soil Sci. Soc. Amer. Proc.37, 220-224.

Olomu MO, Raca GJ, Cho CM (1973), Effect of flooding on Eh, pH and concentration of Fe and Mn in several Manitoba soils. Soil Sci. Soc. Amer. Proc.37, 220-224.

Patrick WH. Jr, Dalaune RD (1972), Characterisation of oxidized and reduced zones in flooded soils. Soil Sci. Soc. Am. Proc. 36, 573-576.

Puente, ME, Bashan, Y. Li Lebsky, VK (2004), Microbial populations and activities in the rhizoplane of

Riley, D. and Barber, S.A. (1969), Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root-induced pH change at root-soil interface. Soil sci. Soc. Amer. Proc. 33, 905-908.

Rovira A D and Campbell R 1974 Scanning electron microscopy of micro-organisms on the roots of wheat. Microbial Ecol. 1, 15–23.

Ruifu Zhang , Jorge M Vivanco , Qirong Shen (2017), The unseen rhizosphere root-soil-microbe interactions for crop production, Curr Opin Microbiol, Jun;37:8-14.

Sarkar AN (1977), Biochemistry and Micromorphology of The Rhizosphere, Ph.D. Thesis submitted to the University of Wales for award of Ph.D. Degree.

Sarkar AN (1980), Rhizosphere and its Effect on the Nutrients Availability of Plants – A Review, Agric. Review 1 (1), pp 1-18.

Sarkar AN, Jenkins DA (1979), Micro- morphology of the Rhizosphere, Indian Society of Soil Sci. Vol. 27 (2): 133-137, 1979.

Sarkar AN, Jones RG Wyn (1982a), Effect of Rhizosphere pH on the availability and uptake of Fe, Mn, and Zn; Plant and Soil (Published in Hague, The Netherlands), 64, p.p.361- 372 (the Paper forms a part of Ph.D. Thesis on Biochemistry and Micro-morphology of Rhizosphere, University of Wales, U.K. in 1977.

Sarkar AN, Jones RG Wyn (1981), Effects of ionic competition in the Rhizosphere on the Phosphate nutrition of dwarf French beans; Crop Management to meet New Challenges, National Symp. at Hissar, India held on March- 14016, 1981 by the Indian Society of Agronomy; Proceedings, pp no. 319-183.

Sarkar AN, Jones RG Wyn (1982b), Influence of Rhizosphere on the Nutrient Status of Dwarf French Beans, Plant and Soil (Published in Hague, the Netherlands), 64, p.p.369- 380 (the Paper forms a

part of PhD. Thesis on Biochemistry and Micro morphology of Rhizosphere, University of Wales, U.K. in 1977).

Sarkar AN, Jenkins DA, Jones GW (1976), Modifications to Mechanical and Mineralogical Composition of Soil within Rhizosphere, International Conference at the Oxford University, U.K in 1976, Also the Proceedings Published in the form of a Book titled " Plant- Root Interface, Elsevier Publication; Edited by Stanley A. Barber, Purdue University, U.S.A.

Sarkar AN, Jenkins DA, Jones GW (1980a), Detection of elemental Concentration Change in Root Environment: I - Contact Micro – Autoradiography, Indian Society of Soil Sci. Vol. 27 (3): 286-294, 1979-137, 1979.

Sarkar AN, Jenkins DA, Jones GW (1980b), Detection of Elemental Concentration Change in Root Environment: II Electron Probe Micro -Analysis, Indian Society of Soil Sci. Vol. 28 (1):42- 48, 1980-p.p. 137, 1979.

Sarkar AN, Jones GW (1980c), Influence of the application from diverse sources of Nitrogen and Phosphorus on Growth and Nutrient Uptake of Dwarf French Beans, Indian Journal of Plant Physiology, Vol. XXIII, No. 3, p.p. 248-256.

Segal E. Kushnir, Mualem Y, Shani U (2008), Microsensing of water dynamics and root distributions in sandy soils, *Vadose Zone Journal,* 1018, 1026

Stoops G, Jongerus A (1975), Proposal for a micromorphological classification of soil materials, i. a classification of the related distributions of fine and coarse particles a reply, Geoderma, 13, 189.

Tardieu F, Simonneau T, Parent B (2015), Modelling the coordination of the controls of stomatal aperture, transpiration, leaf growth, and abscisic acid: update and extension of the Tardieu-Davies model, *Journal of Experimental Botany,* 66, 2227- 2237.

Toal ME, Yeomans C, Killham K, Meharg AA (2000), A review of rhizosphere carbon flow modeling, *Plant and Soil,* 222, 263–281.

Venant Nihorimbere, Marc Ongena, Maïté Smargiassi, Philippe Thonart (2011), Beneficial effect of the rhizosphere microbial community for plant growth and health, Biotechnol. Agron. Soc. Environ. 2011 15(2), 327-337.

York, Larry M, Andrea Carminati, Sacha J. Mooney, Karl Ritz, Malcolm J. Bennett (2016), The holistic rhizosphere: integrating zones, processes, and semantics in the soil influenced by roots, Journal of Experimental Botany, Volume 67, Issue 12, June 2016, Pages 3629 643, https://doi.org/10.1093/jxb/erw108