CHAPTER 1

General Introduction
INTRODUCTION AND REVIEW OF LITREATURE

Use of fertilizers for different crops on the basis of individual crop response to their direct application without considering the residual effects of fertilizers applied to the preceding crop may result in the application of relatively high and some times uneconomic dose of fertilizers. The farmers grow wheat (*Triticum aestivum* L. emend. Fiori and Paol.) after Paddy and this rotation is popular wherever irrigation facility is available in Bundelkhand region, Vertisols and Ustochrepts of arid, semi-arid and subhumid regions. Cultivation of wheat after Paddy results in economy of about 35 kg N/ha compared with growing wheat after cereals (Singh and Sahu 1981). Hence a study was undertaken to find out the N requirement of in Paddy-wheat cropping system.

Utilization and fate of fixation products

The availability of isotopically labelled $^{15}$N$_2$ provided a mechanism for tracing the path of nitrogen in the fixation process. The initial product was found to be NH$_3$. However, this ammonia rapidly becomes incorporated into organic combination in amino acids and amides. The carbon skeletons required for the formation of the amino
acids are presumably derived from the respiratory metabolism of carbohydrates. The prime point of ammonia entry into organic combination was thought to be via condensation with α-ketoglutarate to form glutamic acid as occurs in higher plants. However, recent evidence from various free-living bacteria, extracts of Rhizobium bacteroids, and blue-green algae indicates the operation of an alternate pathway. Ammonia is initially converted to glutamine by the enzyme glutamine synthetase. This glutamine is the delaminated in the presence of α-ketoglutarate by the recently identified enzyme glutamate synthetase, with the resulting formation of glutamate.

\[
\text{NH}_3 + \text{glutamate} + \text{ATP} \xrightarrow{\text{glutamine synthetase}} \text{glutamine} + \text{ADP} + \text{Pi}
\]

\[
\text{Glutamine} + \alpha\text{-ketoglutarate} + \text{NADPH}^+ (\text{NADH}^+) \xrightarrow{\text{glutamate synthetase}} 2 \text{glutamate} + \text{NADP(NAD)}
\]

**Nitrification**

Ammonia can be released into the soil either as a result of N\textsubscript{2} fixation by free-living microorganisms or from the decomposition of dead organisms. This ammonia, as discussed later, can be taken up directly by plants or it may be oxidized to nitrate and nitrite. This biological oxidation of ammonia to nitrite and nitrate is termed nitrification and is catalysed by bacteria and some fungi. The oxidation of ammonia is achieved by bacteria belonging to the Nitroso group of genera which
convert ammonia from the -3 oxidation state of nitrogen to the +3 situation of nitrite. The oxidation is assumed to occur via 2 electron steps with the production of intermediates of -1 and +1 oxidation states.

On the evidence that intact \textit{Nitrosomonas} cells would not oxidize ammonia under anaerobic conditions and that hydroxylamine and not nitrite accumulates when cells are treated with hydrazine, it appears that the first stage of nitrification involves the oxidation of ammonia to hydroxylamine.

\[
\text{NH}_3 + \frac{1}{2} \text{O}_2 \rightarrow \text{NH}_2\text{OH} \\
\text{Hydroxylamine}
\]

The mechanisms of this oxidation are not known at this time. It has not been possible to demonstrate ammonia oxidation by cell-free extracts; however, in view of the fact that ammonia oxidation by intact cells is inhibited by thiourea and chelating agents, it is possible that a copper containing oxidase may be operative.

\textbf{Hydroxylamine to nitrite}

\textit{Intrac} \textit{Nitrosomonas} can readily oxidize hydroxylamine to nitrite. This oxidation can also be demonstrated in cell-free extracts of the organism provided that a suitable electron carrier such as cytochrome \textit{c} is present. This cell-free oxidation is stimulated by flavine mononucleotide (FMN) and inhibited by atabrine and cyanide and
carbon monoxide. In addition, it can be demonstrated that the metabolism of hydroxylamine by intact cells is associated with changes in the oxido-reduction status of cytochrome \( b \), \( c \), and \( a \). On the basis of this evidence, it is considered that the metabolism of hydroxylamine involves a dehydrogenation with a transfer of electrons through a flavor protein to the cytochrome electron transport chain.

\[
\begin{align*}
\text{NH}_2\text{OH} & \rightarrow \text{FLAVIN} \rightarrow \text{cyt} \; b \rightarrow \text{cyt} \; c \rightarrow \text{cyt} \; a \rightarrow \text{O}_2 \\
\text{ATABRINE} & \quad \text{CN} \\
& \quad \text{CO}
\end{align*}
\]

The product arising from the dehydrogenation of hydroxylamine has not been isolated or characterized, but is believed to be nitroxylnitroxyl (NOH) in which the nitrogen atom is in the +1 oxidation state. Under aerobic conditions, the nitroxylnitroxyl can be converted to nitrite. According to Aleem et al., this conversion is achieved in a semi-cyclic process involving the oxidation of a further intermediate nitrohydroxylamine, \( \text{NO}_2\text{NHOH} \). The oxidation of nitrohydroxylamine can be demonstrated using cell-free extracts. Oxidation occurs in the absence of added electron acceptors, and although cytochrome \( c \) is reduced during the oxidation of hydroxylamine the electron carrier is not reduced during the stoichiometric conversion of nitrohydroxylamine to nitrite. Although nitrohydroxylamine has not as yet been identified as an intermediate of hydroxylamine oxidation, the studies of Aleem convincingly imply its functioning in the sequence outlined below:
\[
\begin{align*}
\text{NH}_2\text{OH} + 2 \text{ cyt c Fe}^{3+} & \rightarrow (\text{NOH}) + 2 \text{ cyt c Fe}^{2+} + 2\text{H}^+ \\
(\text{NOH}) + \text{HNO}_2 & \rightarrow \text{NO}_2\cdot\text{HNOH} \\
\text{NO}_2\cdot\text{HNOH} + \frac{1}{2}\text{O}_2 & \rightarrow 2\text{HNO}_2 \\
2 \text{ cyt c Fe}^{2+} + 2\text{H}^+ + \frac{1}{2}\text{O}_2 & \rightarrow 2 \text{ cyt c Fe}^{3+} + \text{H}_2\text{O}
\end{align*}
\]

Sum \[\text{NH}_2\text{OH} + \text{O}_2 \rightarrow \text{HNO}_2 + \text{H}_2\text{O}\]

**Nitrite to nitrate**

The conversion of the nitrite, arising from the activities of the Nitroso group of genera, to nitrate involves the oxidation of nitrite. Bacteria belonging to the Nitro group of genera *Nitrobacter* and *Nitrocytis* are capable of this conversion.

In intact *Nitrobacter* treated with nitrite, cytochrome c and cytochrome oxidase-like components become reduced suggesting that nitrite oxidation requires the participation of an electron carrier cytochrome system. Such an oxidative system can also be demonstrated using cell-free cytochrome electron particles (nitrite oxidase) prepared from *Nitrobacter*, which can transfer electrons from nitrite to molecular oxygen according to the scheme.

\[
\text{NO}_2 \rightarrow \text{cyt a}_1 \rightarrow \text{cyt a}_3 \rightarrow \text{O}_2
\]

Since nitrite oxidation is mediated by the electron transfer chain, the terminal acceptor oxygen must be reduced to water; however, clearly nitrite, the substrate which apparently stimulates electron flow,
has no electrons to donate. Accordingly, it is suggested that the true substrate for nitrite oxidase is a hydrated form of nitrite and thus the oxidation of the nitrite molecule is achieved at the expense of an oxygen atom from water and not from atmospheric oxygen.

This feature was elegantly demonstrated by the use of $^{18}$Oxygen and H$_2$O. When nitrite is oxidized in the presence $^{18}$O$_2$ little of the isotope is recovered in nitrate. If the oxidation occurs in H$_2$O the nitrate produced is extensively enriched in $^{18}$O. Furthermore, the oxidation of nitrite to nitrate can occur in intact cells-free extracts in the presence of an artificial electron acceptor in the absence of oxygen. Thus, it is convenient to depict the oxidation of nitrite as follows:

$$\text{NO}_2^- + \text{H}_2^{18}\text{O} \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$$
$$\text{NO}_2^- + \text{H}_2^{18}\text{O} + 2 \text{cyt a}_1\text{Fe}^{3+} \rightarrow \text{N}^{18}\text{O}_3 + 2 \text{cyt a}_1\text{Fe}^{2+} + 2\text{H}^+$$
$$2 \text{cyt a}_1\text{Fe}^{2+} + 2\text{H}^+ + \frac{1}{2}\text{O}_2 \rightarrow 2 \text{cyt a}_1\text{Fe}^{3+} + \text{H}_2\text{O}$$

**Sum** $\text{NO}_2 + \frac{1}{2}\text{O}_2 \rightarrow \text{NO}_3 + \text{Energy}$

**Chemosynthesis**

The nitrifying bacteria are chemosynthetic autotrophs deriving the energy required for their biosynthetic synthesis from the oxidation of ammonia and nitrite. From the schemes outlined above, it is clear that energy derived from the oxidations could be conserved as the high energy bonds of ATP which could be generated as electrons flow
through the cytochrome components of the electron transfer chain. The cytochrome electron transport system of *Nitrobacter* is somewhat peculiar in that during the oxidation of nitrite phosphorylation is achieved without the involvement of cytochrome c.

\[
\text{H}_2\text{O.NO}_2 \rightarrow \text{cyt a}_1 \rightarrow \text{cyt a}_3 \rightarrow \text{O}_2
\]

In *Nitrosomonas*, ATP is generated at conventional locations in the electron transfer chain between cyt b and cyt c and during the oxidation of reduced cyt c by cytochrome oxidase.

\[
\text{NH}_2\text{OH} \rightarrow \text{cyt b} \rightarrow \text{cyt c} \rightarrow \text{cyt oxidase} \rightarrow \text{O}_2
\]

The fixation of carbon dioxide carried out by these autotrophs, in addition to ATP, requires reduced pyridine nucleotides. However, as depicted, the oxidation of ammonia by the Nitroso group or nitrite by the Nitro group does not result in the generation of reductant. Instead, it appears that reductant is generated by energy-linked reversed electron flow in which NAD reduction is achieved at the expense of ATP during the oxidation of ammonia or nitrite.
Clearly, the bioenergetics and chemosynthesis of *Nitrobacter* and *Nitrosomonas* pose extremely complex problems with regard to energy budgets; some of these are discussed at length by Gibbs and Schiff, Aleem, Wallace and Nicholas.

**Denitrification**

The nitrate produced as a result of the activities of the nitrifiers *Nitrosomonas* and *Nitrobacter* can be taken up by higher plants and further metabolized. Additionally, however, the nitrate can be converted to nitrogen gas or nitrous oxide, or a mixture of these two gases in a process termed denitrification. The gases return to the atmosphere and thus denitrification represents a mechanism by which soil nitrogen is depleted.

Characteristically the denitrifying organisms are bacteria which can facultatively utilize nitrite or nitrate rather than oxygen as the terminal hydrogen acceptor in the energy-yielding electron transfer. Denitrification occurs most readily under anaerobic conditions. The
process is inhibited by oxygen because this gas effectively competes with nitrite or nitrate as the terminal electron acceptor.

The first stage in denitrification involves the reduction of nitrate to nitrite. The enzyme involved in this reaction has been termed respiratory nitrate reductase and is, in contrast to the assimilatory nitrate reductase, a particulate enzyme. Particulate respiratory or dissimilatory nitrate reductase has been prepared from a variety of organisms and it can be demonstrated that the conversion of nitrate to nitrite is coupled with the generation of ATP. The transfer of reductant in these nitrate reductases appears to be mediated by cytochromes and molybdenum.

Generalized scheme for respiratory nitrate reductase:

\[
\begin{align*}
\text{Substrate} & \rightarrow \text{reduced} \rightarrow \text{FAD} \rightarrow \text{cytochrome} \rightarrow \text{cytochrome} \\
& \quad | \quad \text{oxidase} \\
& \quad \text{Nitrate reductase} \quad \downarrow \\
& \quad \text{NO}_3^- \quad \downarrow \\
& \quad \text{O}_2 \\
& \quad \text{NO}_2^-
\end{align*}
\]

Nitrite reduction

The nitrite produced can be further metabolized to nitrogen or nitrous oxide by a variety of microorganisms. The sequence of intermediates and enzymes involved in these conversions are still incompletely resolved.
By utilizing whole cells and a suitable respiratory substrate, it can be demonstrated that under anaerobic conditions nitrite can be converted to nitrogen gas. The production of nitrogen gas by the intact cells can be prevented by azide but there is a continued release of nitrous oxide. These observations suggest that nitrous oxide may be a key intermediate in the conversion of nitrite to gaseous nitrogen. In some organisms, *Corynebacterium nephridii* for example, nitrite reduction appears to proceed only as far as nitrous oxide. Nitrogen gas or nitrous oxide production is stimulated in many instances by the addition of hydroxylamine. This observation raises the possibility that the metabolism of nitrite by these denitrifying organisms involves hydroxylamine as an intermediate. However, current ideas favour the concept that hydroxylamine is not a functional intermediate in the conversion but can interact with as yet unidentified products to enhance gas production. The sequence of reactions involved has been proposed as follows:

\[
\begin{align*}
\text{HNO}_2 & \xrightarrow{\text{Denitrifying enzyme}} \text{NO} \xrightarrow{\text{Nitric oxide reductase}} \text{N}_2\text{O} \xrightarrow{\text{Nitrous oxide reductase}} \text{N}_2
\end{align*}
\]

The initial metabolism of nitrite has been demonstrated in cell-free extracts and is mediated by an enzyme variously termed denitrifying enzyme or nitrite reductase. In certain species the enzyme
contains cytochromes with two haem derivatives whereas in other species the enzyme is apparently a copper-containing protein. The products of nitrite reduction by the cell-free extracts have been identified as nitric oxide.

The nitric oxide can be further metabolized to nitrous oxide by a particulate fraction under anaerobic conditions in the presence of lactate. However, the electron donor component at this reduction step has not as yet been identified.

The subsequent conversion of nitrous oxide to nitrogen has not been achieved in cell-free system. However, the observed sensitivity of the reduction to azide in intact cells appears to demonstrate the functioning of metal ions in this step.

**The entry of ammonia into organic combination**

Detailed investigations by Yemm and Willis indicated that the respiratory metabolism of roots of barley (*Hordeum vulgare*) was markedly influenced by nitrogen metabolism. Transfer of excised nitrogen-deficient roots to ammonium salts, or nitrate or nitrite resulted in a rapid increase in CO₂ evolution and increased oxygen uptake.

Similar respiratory changes are observed when nitrogen-deficient *chlorella* is transferred to medium enriched with ammonia, nitrate or nitrite. In the studies of barley utilizing ¹⁵N isotopic nitrogen it was established that the high rates of respiration encountered during nitrogen nutrition were associated with a rapid synthesis of nitrogen
compounds in the tissue. A massive accumulation of the amides glutamine and asparagines was accompanied by a rapid loss of tissue carbohydrate. The glutamine was labeled in both the amino and amide group; aspartic acid and asparagines, although labelled, had a much lower abundance of $^{15}$N than glutamine and it was thus considered that they were not primary products of nitrogen assimilation.

The physiological observations can be adequately accounted for with the demonstration of enzymes capable of catalyzing the appropriate syntheses. Until recently it was considered that the principal and possibly only modes of entry of ammonia into organic combination in higher plants were through glutamine and glutamate involving the activities of glutamine synthetase and glutamic dehydrogenase.
Fig. 1 The effect of different sources of nitrogen on carbon dioxide production by 14-day-old barley seedlings. Sodium nitrate O—O; sodium nitrite Δ—Δ; ammonium dihydrogen phosphate ▼—▼; culture solution lacking nitrogen •—•.
The incorporation of ammonia into glutamine is mediated by the enzyme glutamine synthetase according to the scheme

\[
\begin{align*}
\text{COOH} & \quad \text{COOH} \\
\text{CHNH}_2 & \quad \text{CHNH}_2 \\
\text{CH}_2 + \text{NH}_3 + \text{ATP} & \quad \text{CH}_2 + \text{ADP} + \text{Pi} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{COOH} & \quad \text{COOH} \\
\text{Glutamate} & \quad \text{Glutamine}
\end{align*}
\]

In the presence of glutamic dehydrogenase ammonia combines with \( \alpha \)-ketoglutarate in a reductive amination to produce glutamate.

\[
\begin{align*}
\text{COOH} & \quad \text{COOH} \\
\text{CO} & \quad \text{CHNH}_2 \\
\text{CH}_2 + \text{NH}_3 + \text{NADH}^+ & \quad \text{CH}_2 + \text{NAD} + \text{H}_2\text{O} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{COOH} & \quad \text{COOH} \\
\alpha \text{-Ketoglutarate} & \quad \text{Glutamine}
\end{align*}
\]

The bulk of the enzyme is located in the mitochondria and utilizes \( \text{NADH}^+ \) preferentially. Additionally, there have been reports of an \( \text{NADPH}^+ \) glutamic dehydrogenase located in the chloroplasts and the enzyme has been suggested to function extensively in ammonia incorporation in leaves. However, the concentration of ammonia
required for glutamate synthesis by the enzyme glutamic dehydrogenase is higher than that normally encountered in plant tissues. In fact the ammonia concentration required for optimal glutamic dehydrogenase activity is sufficient to uncouple photophosphorylation. These observations tend to preclude, but do not eliminate, glutamic dehydrogenase as a major route of entry of inorganic nitrogen into organic form.

Recent evidence suggests that the problem of high ammonia requirements is circumvented by the utilization of glutamine as the nitrogen donor in glutamate synthesis in a reaction catalysed by glutamate synthetase (glutamine oxoglutarate amino transferase). The enzyme from carrot (*Daucus carota*), sycamore (*Acer pseudoplatanus*) cell cultures and pea (*Pisum sativum*) roots is able to utilize NADH⁺ or NADPH⁺ as the reduced pyridine nucleotide and asparagines as well as glutamine can function as the amide donor. In contrast the enzyme characterized from chloroplasts requires reduced ferredoxin.

Thus an alternative and perhaps preferred method of entry of inorganic ammonia nitrogen into organic combination is through the combined activity of glutamine synthetase and glutamate synthetase. Such a system is comparable to the scheme described for the entry of ammonia into organic combination in nitrogen fixing organisms.

Although there are other reports of the combination of ammonia with pyruvate and oxaloacetate to produce alanine, aspartate and ammonia to give asparagines.
The essentiality of several elements becomes quite obvious when one studies the molecular structure of the various compounds found in living cells. The main structural components of protoplasm are the protein molecules, which contain carbon, oxygen, hydrogen, nitrogen and frequently sulphur and phosphorus. Most of the rather complex molecules with in a cell contain carbon, hydrogen and oxygen atoms, but in addition to these atoms, the chlorophyll molecule contains magnesium, the middle lamella consists primarily of calcium pectate and certain respiratory enzymes contain iron or copper as part of their prosthetic groups. Without any one of these component atoms, gross abnormality develops. Only ten elements (C, H, O, N, S, P, Mg, Ca, Fe and Cu) have been shown to be component parts of the cellular molecules.

However, recent evidence has been shown that certain elements are essential for the normal development of plants because the ions concerned (e.g. Mg++, Mn++ etc.) are necessary for the functioning of various enzyme systems. Broadly speaking, these elements are classified into three groups viz. 'macro', 'micro' and 'ultramicro' nutrients. The elements which are required by plants in concentrations exceeding one part/million or often ten times more, are called 'macro nutrients' (C, H, O, N, S, P, Ca etc.) whereas the elements required in low concentration i.e. less than 1 ppm. are placed in the category of 'micro nutrients' (Cu, Zn, Fe, Mn etc.). The term 'ultramicro nutrients' denote those elements which are required in very low concentration i.e.,

(16)
one part/billion or less under which comes Mo and Co. These elements are mostly absorbed as ions through plant roots from the soluble salts present in the soil.

Therefore by the constant use of soil in growing the plants the soil is likely to become deficient in some of the essential nutrients. In order to make up such deficiencies of these nutrients, salts of these elements are supplied in the dissolved state in the form of fertilizers.

In literature, it has been shown that fertilizers not only make up the deficiency of the nutrients but affect the yield or growth of the plant and have an appreciable effect on the chemical composition of the plants and their products. This effect has been shown to be governed by other important factors e.g. level or dose of fertilizer, extent of irrigation, basal richness of the soil, associative effect of fertilizers in their different combinations forms, modes and sources of fertilizers, time of application, stage of cutting, progress of maturity etc. Among all the fertilizers, nitrogen and trace elements have been shown to play a special role in plant physiology. A short review of the same is presented below under the following headings.

**Nitrogenous fertilizers**

Nitrogen is an essential nutrient for plants as it is a constituent of protoplasm of every living cell and is needed in large amounts for all growth processes. The adequate supply of nitrogen increases yield, stimulates growth and develops resistance to diseases. It is utilized in
the formation of chlorophyll, vitamins, hormones, amino acids, amides, proteins alkaloids etc. in plants. However its deficiency affects adversely chlorophyll of the leaves, growth and yield of plants and their products.

In order to maintain and/or improve the usefulness of the soil it is necessary to ensure adequate level of nitrogen by applying nitrogenous fertilizers from time to time. A large number of workers have shown that the application of nitrogenous fertilizer has an appreciable effect on the yield and chemical composition of the plants and their products (proteins, amino acids, fat, carbohydrates, Cu, Zn, Mn, Fe etc.) and a brief account of these studies is given in the following paragraphs.

**Affect over crude protein content**

It has been commonly observed that in most of the cases the application of N-fertilization had a beneficial effect on crude protein content. An increase has been reported by Upendra et. al. (1999) : Kaul & Raghavish (1975) : Krishanasami et.al. (1975) : Eggum & Juliano (1975) in rice grain; Derzhavin et. al. (1977) : Austin & Ahuja (1974) : Sawhney & Stoyanov (1974) in wheat; Korekov & Orlova (1977) in broad bean, barley, Rye & clover : Singh & Prasad (1977) in maize and by Saini J.P. et. al. (1999) in rice. Besides this, it has been shown by a number of workers that this effect of fertilizer is governed by other important factors like level, dose or mode of fertilizer and stage of application, forms of fertilizers, irrigation rain-fall soil and temperature.
Mahapatra B. S. et al. (1996) found a level of 90 kg N/ha more beneficial than other levels tested in increasing the yield of rice. Likewise Govindaswami et al. (1973) found a dose of 80 kg/ha more effective in the case of rice crop.

In different experiments, beneficial effect of nitrogen fertilization in increasing the crude protein content was reported by Suzuki et al. (1977) with top dressing at full heading stage; Kudzin et al. (1976) with preharvest foliar application of 20%-30% urea; Gupta & Gupta (1975) with combined basal and foliar application; Nishizawa et al. (1977) with spraying of urea at heading stage; Hsieh et al. (1975) with application of nitrogen at panicle initiation stage whereas Datta et al. (1972) preferred split applications i.e. half nitrogen applied at planting and half at heading stage over others.

It is evident from available literature that the form of the fertilizer plays a significant role in increasing the crude protein content of plant and their products. Datta et al. (1972) found benzonare, tenoran, CP 17029 better than urea in rice crop. Zrazhevshii (1974) preferred a mixture of urea and ammonium nitrate than others and Cencelj & Jelenic (1968) while comparing the efficiency of (NH₄)₂SO₄, NH₃NO₃, CaCN₂, NaNO₃ and urea as fertilizer found that urea and NaNO₃ were the best and CaCN₂ was least effective in increasing the crude protein content of silage corn.

Irrigation, rain-fall and maturity have also been found to affect the protein content of rice grain (Socorro & Sanzo 1976; Taira 1971). Juliano
et. al. (1964) reported a significant effect of season on the protein content of rice. Baba (1971) and Hiremath S.M. et. al. (1998) reported that protein content differed in varieties of rice even when they were applied the same dose of N-fertilizers, showing that each variety required different amount of N-fertilizer.

**Effect over non-protein nitrogen content**

Non-protein nitrogen gives an idea of that amount of nitrogen present in plant which is not utilized in the protein synthesis. An increase in the non-protein nitrogen content has been shown in experiments on rice by Dutta & Barua (1976), on barley by Barazczak (1977), on wheat by Biskupski & Zych (1975); Volleidt & Myadelets (1977); Biskupshi & Wawry Susk (1972) and on Italian rye grass by Mahapatra B.S. et. al. (1996) with the application of large doses of N-fertilizers.

**Effect over the total free amino acid content**

A perusal of literature shows that the effect of N-fertilization on amino acid content is variable.

While working on wheat grains Tolochko & Zagorcha (1974) and on rice grain Houston et. al. (1969) reported that there is no effect of N-fertilization on amino acid content. However a slight increase in the amino acid content in barley was observed by Andriesh & Gozhinetshaya (1976), in hay by Cillirs et. al. (1975) and in wheat grains by Austin & Ahuja (1974). An increase in lysine content of different
varieties of rice except basmati-370 due to increase in protein content with nitrogen fertilization has also been reported by Chavan & Duggal (1978), Hsieh et. al. (1975) found that the application of nitrogen at panicle initiation stage led to increased contents of glutamic acid, leucine, tyrosine, histidine, phenyl alanine and decreased contents of lysine, serine, glycine, alanine and threonine. (Sasada, Y. et. al. 1983; Freman H. C. et. al. 1970; Aleksandrow G. G. et. al. 1977).

Effect over ether extract

Like amino acid here also the effect of N-fertilization on ether extract was reported to be variable. Heavy rate of nitrogen application was shown to reduce the fat content in rice grains during ripening period by Yanatory & Kido (1973) and in rape seeds by Mazur et. al. (1977). However an increase in the fat content in grass was reported by Ikeda et. al. (1976). Also a slight increase in fat content was observed by Dutta & Barua (1978) in high yielding variety of rice; by Singh S.P. et. al. (2001) in soyabean.

Effect over mineral contents

A survey of literature showed that calcium and phosphorus contents are affected with increasing N-fertilization. A decreasing trend in calcium and phosphorus was reported by Khan & Pathak (1976) reported an increase in phosphorus and decrease in calcium content on alfalfa grass. Rinne & Takala (1971) found no effect on phosphorus and calcium.

(21)
contents of pastures with nitrogen fertilization. However Penk (1977) observed a decrease in the mineral content in sayabean. El Leboudi et al. (1975) reported that the N-application at a depth of 10 cm below the level of the soil was beneficial in increasing phosphorus content of rice grain. Samrath Lal Meena et al. (2002) found a decrease in calcium and magnesium and no effect on phosphorus content of meadow pasture species with nitrogen fertilization.

**Effect over total carbohydrate**

In their experiments, Reid & Strachan (1974) on rya grass, Sutoh et al. (1972) on oat crop, reported a decrease in carbohydrate content with increasing nitrogen fertilization while Krishnasamy et al. (1975) found the carbohydrate content of rice grain to be independent of the application of N-fertilizer.

**Effect over trace elements**

Realising the importance of trace elements in the nutrition of human beings and animals, a number of workers studied the effect of N-fertilization on the trace elements. Jha et al. (1975) found foliar spray as useful for increasing the uptake of iron and manganese whereas Kausar et al. (1976) reported an increase in the copper uptake of rice in calcareous soil with N-application. Likewise an increase in zinc, manganese and iron contents with nitrogen fertilization was reported in their experiments by Gyory (1975) over wheat and corn, Rodriguez &
Rhivera (1976), Gzuba & Murzynski (1976), Falkowski et. al. (1977) and Knabe et. al.(1964) on grasses and hay. Subrahmaniyan K. et. al. (1999) reported an increase in zinc and copper contents and a decrease was observed in manganese and iron contents with increasing N-fertilization on grasses.

**Micronutrients used as fertilizers**

It is an established fact that micronutrients like copper, boron, zinc, manganese, iron, molybdenum and chlorine are very essential for the proper growth of plants. Recently the importance of Co, Na, Ni, Ge, V has also been realized in the physiological functioning of plants and therefore they have been included in the list of micronutrients. Amongst these elements zinc is known to act catalytically in various reactions and photosynthesis. Copper which was regarded formerly as a plant poison but now its foliar spray is used to control the diseases of citrus family. It takes part in the formation of iron porphyrin, a precursor of chlorophyll which showed the role of copper in its molecule. Boron is found to be essential to reduce nitrates to ammonia in plants, prior to the synthesis of proteins and amino acids. Recently chlorine in the form of sodium chloride is found to be useful for the rice crop. Thus, realizing the great importance of these trace elements in plant metabolism, several workers studied the effect of their application as fertilizer on the chemical composition of plants and their products. A brief resume of the same is given in the following lines.

(23)
Effect over crude protein

It is a well known fact that trace elements when applied in micro
doses, have a beneficial effect on the crude protein content of plants.
Gyul'akhmedov et. al. (1977) in his experiment on wheat with Mo; Rao
(1971) on onions: Vasayaev & Shidlovshaya (1975) on grass have
reported an increase in crude protein content with the application of
micronutrients. Quite contrary to general trend Kevorkov & Tishchenko
(1975) found no effect and whereas Ter-Arakelyan & Kazanchyan (1977)
observed an adverse effect on crude protein content with the
application of trace minerals.

Kozel (1977) reported that powdering the seeds of rice and
mixing with sulphates of manganese, cobalt, copper, magnesium and
molybdenum increased the crude protein of the grain. The same
technique of powdering of seeds of wheat with trace elements was
reported by Merzlikin & Koroleva (1977) on protein content of grain.

In the case of trace elements the dose of fertilizer is of prime
importance. Miskovic & Jevatic (1973) found small doses quite effective
in increasing the crude protein content of stems and leaves of leucerne,
whereas the large doses had an adverse effect.

Besides this, Singh et. al. (1978) found foliar application of
micronutrients beneficial in increasing the crude protein content in
comparison to other methods of application. Basal richness of soil has
also been shown to have some role in trace mineral fertilization.
Kumaresn M.A. et. al. (2001) grew maize in trace mineral deficient soil and reported that the application of deficient minerals resulted in increasing the crude protein from 8 to 19%.

**Effect over non-protein nitrogen and total free amino acid content**

Vasyaev & Shidlovekaya (1975) have reported an increase in non-protein nitrogen content of grass while Beres & Tatar (1977) found total free amino acid content of mature potato tubers to increase with the application of trace elements. However a decrease in total free amino acid content was observed by Sharma P. K. et. al. (1999) in millet leaves.

**Effect over ether extract and total carbohydrate**

An increase in fat content was reported by Gyul'akhmedov et. al. (1977) in cotton seed by the application of trace elements.

Stolyarov (1977) while working on potatoes and Dobrolyubskii & Matyushenko (1970) on corn have reported an increase in the total carbohydrate content while Ter-Arakelyan & Kazanchyan (1977) and Krotkikh & El'kina (1975) observed a decrease in sugar content of tobacco and wheat respectively by the application of trace elements.

**Effect over calcium and phosphorus contents**

The study of the available literature revealed that the calcium content is not affected consistently by the application of trace elements. However, an increase in calcium content with cobalt was noticed by
Kuczynska & Sapec (1976) in one year out of two years project on grassland, while Miskovic & Jevtic (1973) observed no regular increase in calcium content of lucerne by the trace elements fertilization.

Phosphorus was found to be affected significantly by the application of trace elements. Ter-Arakelyan & Kazanchyan (1977) observed an increase in phosphorus content of the tobacco leaves by the application of trace elements. Moisture-zinc and moisture-iron interactions were found to influence phosphorus content significantly in rice as reported by Pathak et. al.(1975). Charvorti A. (2001) showed a decrease in phosphorus content in grape wine shoots and Shankaralingappa B.A. et. al. (2000) obtained similar results in tomato leaves by the trace element fertilization.

**Effect over micro elements**

A perusal of literature revealed that the application of trace elements have a correlation with the trace elements supplied and absorbed by the plants and their products. Jha et. al. (1975) have shown that foliar spray of Fe and Mn increased the uptake of these elements in rice grain and straw. An application of Mn, Zn, Mo and B increased the uptake of these elements in rice grain (Kevorkov & Tishchenko, 1975). Iron application significantly increased the iron content but did not affect manganese content at any stage of growth whereas manganese application significantly decreased iron and increased manganese contents at all stages in paddy roots (Singh & Singh, 1975). Pathad et. al.
(1975) found that the uptake of Fe, Zn and Mn was significantly increased in rice by the application of iron and zinc. While working on hay, Sapek et. al. (1976) demonstrated an increase in Cu, Co, Mo by the application of these elements. Kuczynska & Sapek (1976) noticed that zinc contents of grassland were decreased by the application of manganese, copper and cobalt.

Further, it was also observed that foliar application of copper sulphate was found effective in increasing the copper content in hay Agrawal S.C. (1964). Similarly Kao & Juang (1977) found foliar application of trace elements superior to that of basal application on sugar cane leaves. Kumar J. S.et. al. (1967) have shown that all the trace elements especially manganese were significantly affected with the application of trace minerals in different varieties of oat, whereas Palaveev et. al. (1975) noted a negative correlation among the micronutrients.

Other factors governing the effect of fertilizers

A perusal of literature revealed that the effect of fertilizers on the chemical composition of plants and their products is governed by some other important factors like mode and stage of application, dose of fertilizers, form of fertilizers, basal richness of soil, rain-fall, temperature, irrigation, variety considered, time of harvesting etc., which seem to play a significant role in the physiology of plants. A brief resume of the same is given below.
Modes and stages of application

It is evident from available literature that the different modes of application viz. foliar, spray, seed treatments, broadcasting, band placement and hole placement at different stages of growth have been shown to be beneficial under certain set of experimental conditions in affecting the yield and chemical composition of plants and their products.

Nishizawa et. al. (1977) sprayed urea on the leaves of rice plant at the heading stage and found an 11% increase in protein content. Suzuki et. al. (1977) reported that top dressing of nitrogen at full heading stage increased glutalin and total N-contents of brown rice. Hsieh et. al. (1975) showed that the crude protein content of rice grain increased by the application of nitrogen at panicle initiation stage. Peisakhov & Statsenko (1976) reported an increase in protein content of wheat grain when nitrogen was applied as top dressing. NPK supplementation with N-top dressing during tillering, shooting and earing and combined with micro nutrients increased the grain yield as well as the protein content. Upendra et. al. (1999). The split applications of ammonium nitrate increased the protein content of wheat (kvaratskheliya & Kakhadze, 1973). Pre foliar application of urea was found to be useful in increasing crud protein of corn (Kudzin et. al. 1976). Neelam Kumar Chopra (2000) found spray and broad casting methods superior than band placement, whole placement in their experiments on kinnow. The treatment of the seeds of bean with ammonium molybdate and manganese sulphate
solutions increased the yield, whereas dry treatment of seeds with trace elements was ineffective (Stakanov, 1975).

**Doses or levels of fertilizers**

It is a common observance that sometimes higher doses are uneconomical and prove injurious to the crop in comparison to smaller doses which are beneficial not only in affecting the yield but also have a significant effect on chemical composition of plants and their products. This fact is borne out from the experiment of Singh & De (1978) who reported a dose of 110 kg/ha N-fertilizer beneficial for wheat production than a dose of 150 kg/ha. Likewise Voskerusa (1975) found better results with 100 kg/ha of urea than 150 kg/ha in winter rape. Da Silva et. al. (1975) reported that the yield is maximum at 80 kg/ha in 10-160 kg N/ha levels.

**Forms of fertilizers**

Sinirmova et. al. (1976) reported an increase in uptake of soil N while comparing the two sources of nitrogen and found that urea was better than ammonium sulphate in rice crop. Similarly ammonia application was found superior than ammonium sulphate on the same crop by El. Leboudi et. al.(1975). Cencelj & Jelenic (1967) compared five sources of nitrogen viz. NH₄NO₃, (NH₄)₂SO₄, NaNO₃, CaCN₂ & urea and obtained highest protein content with urea and NaNO₃.

(29)
Effect of season, environment and temperature

They also seem to play an important role in the physiology of plants. While comparing the two seasons, Socorro & Sanzo (1976) revealed that the requirement of NPK for IR-160 variety of rice in dry season was 120-160 kg N/ha whereas in wet season it was only 80-120 kg N/ha. Juliano (1964) reported a difference of 4% protein in the samples of rice planted in different seasons. Nikitina & Volod’ko (1976) found the autumn application of fertilizers better for increasing the starch content of potatoes.

Eggum & Juliano (1975) reported an increase in rice protein content from 8.14% to 9.90% at 12% moisture level. Likewise Ostrovlyanchik & Kondratev (1973) found an increase in protein content of spring wheat with fertilization during normal moisture but there was no effect in dry years.

Effect of irrigation and rain-fall

Irrigation and rain-fall have also been shown to have an important effect on the fertilizer treatments. Chattopadhyay (1975) found the N-up take greater under high irrigation in rice crop whereas Tiara (1971) noticed the variation in protein content of *Oryza-japonica* type of rice with irrigation, variety and maturity. Also increased nitrogen utilization with irrigation was reported by Parihar S.S. et. al (2003) in wheat and barley.

(30)
Effect of variety

It has been observed that under identical conditions of experiment, it is not possible to get the similar results from different varieties of the same species. Singh & Modgal (1978) reported on the basis of two years project that yield and protein content were always higher in bala variety of rice than padma. Baba (1971) estimated the protein content of forty varieties of rice grown under similar treatments and found a variation in protein content from 6.56%-12.86%. Witoszek (1975) studied the protein and non-protein contents of potato tubers and concluded that these fractions depended more on variety of potatoes than the amount.

Effect of soil

It is a well known fact that the characteristics of soil play a vital role in governing the effect of fertilizers on chemical composition of plants. Ghosh et. al. (1976) while experimenting with rice on different soils of Cuttuck & Mandya found the samples of Mandya better in protein content than those from cuttuck. Similarly Lebedeva et. al. (1977) found the protein content to increase on soil with pH 5 in broad bean, wheat etc. than in more acidic soil. An increase in the yield of onions was reported by Downes (1959) on sandy soil. Knabe et. al. (1964) found that the copper content decreased in sandy soil and remain unchanged in fen soil. Khan & Pathak (1976) reported that the potassium

(31)
content increased in sai uplands, than other soils with increasing level of nitrogen on IR-8 variety of rice.

The study of available literature reveals that the experimentation has been done only to increase the yield of food grains like rice, which is a staple diet of large number of Indian peoples but no efforts seem have been made to study the effect of fertilizer on the nutritional aspect of the food grains in India and particularly in this Bundelkhand region of Indo-gangatic plain. It will be of no use to obtain higher yields with lower nutritional value i.e. lesser amounts of protein, fat and carbohydrate. With this aim and object in view, an attempt has been made through the present project, to find a correlation between the elements supplied in the form of fertilizer and the nutrients of rice grain and husk. This project consists of the following experiments.

(a) Effect of levels of N-fertilizers
(b) Effect of trace minerals & Iron pyrites
(c) Studies of bio-chemical

The results obtained from these experiments are likely to furnish information, fresh and useful, for the benefit of research workers as well as for agriculturist of Bundelkhand region of Uttar Pradesh in India.

Rice (*Oryza sativa* L.)-cropping system is commonly followed by the farmers in U.P. Farmers are often constrained in applying recommended doses of N, P and K due to their poor economic condition. Of late, fertilizer recommendation are being developed for a cropping system as a whole. Since information regarding fertilizer need
of rice cropping system is meagre, an experiment was conducted on this aspect.

The experiment was conducted during 2005 at the Research Farm of the college with 9 treatment combinations of 3 N, P and K levels, viz. 120, 90 and 60 kg/ha of recommended doses, applied. The experiment was laid out in randomized block design with 3 replications. The initial soil-available N, P and K were 182, 42 and 210 kg/ha respectively. The soil was basic with pH 7.4. The plant samples were collected at harvest and analysed by the standard methods.

Direct application of 120 kg N/ha recommended N, P 40 and K 40 dose produced significantly highest grain yield of both the crops (Table 1.1). In general, reduction in the N, P and K levels to 120, 90 or 60 kg N/ha of the recommended doses to any crop decreased the grain yield. This may be attributed to fact that rice being an exhaustive crop needs direct application of full recommended doses of N, P and K.
Table 1.1

Effect of different N, P and K levels applied to rice on the uptake (kg/ha) of N, P and K at harvest

<table>
<thead>
<tr>
<th>Treatment (recommended dose of NPK)</th>
<th>Grain yield (tonnes/ha)</th>
<th>Straw yield (tonnes/ha)</th>
<th>Uptake (Kg/ha)</th>
<th>Uptake (Kg/ha)</th>
<th>Uptake (Kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grain</td>
<td>Straw</td>
<td>P</td>
<td>Straw</td>
<td>K</td>
</tr>
<tr>
<td>120 kg/ha</td>
<td>2.29</td>
<td>2.56</td>
<td>22.73</td>
<td>13.34</td>
<td>6.91</td>
</tr>
<tr>
<td>90 kg/ha</td>
<td>2.14</td>
<td>2.52</td>
<td>21.40</td>
<td>11.87</td>
<td>6.15</td>
</tr>
<tr>
<td>60 kg/ha</td>
<td>2.04</td>
<td>2.29</td>
<td>19.95</td>
<td>9.80</td>
<td>5.49</td>
</tr>
<tr>
<td>CD (P = 0.05)</td>
<td>0.82</td>
<td>0.79</td>
<td>0.87</td>
<td>1.30</td>
<td>0.19</td>
</tr>
</tbody>
</table>