STUDIES ON THE MEASUREMENT AND AVAILABILITY OF MAJOR PLANT NUTRIENTS IN FARM ANIMAL WASTES.

MUHAMMAD AYYAZ KHAN
M.Sc (Hons) Agriculture
University of Agriculture Faisalabad
(PAKISTAN)

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AGRONOMY
Agriculture, Food and Environmental Chemistry
Department
University of Glasgow
UNITED KINGDOM

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

In the Name of ALLAH

"Most Gracious, Most Merciful"

"He Who taught (the use of) the Pen,"

"Taught man that which he Knew not."

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SUMMARY

The work of this thesis covers analytical aspects of the determination of ammonium-N using the Technicon AutoAnalyzer II, evaluation of farm animal wastes - algal pond effluent (Scottish Agricultural College, Auchincruive, Ayr) and farmyard manure (Pakistan).

Organic compounds such as amino acids caused positive and negative interferences in the determination of ammonium-N by the Berthelot colour reaction. The reaction conditions such as reagent age, temperature and hypochlorite concentration had effects on the chemistry of colorimetric reaction. A stable room temperature of 25°C and 50 ml l^{-1} hypochlorite reagent are important to achieve consistent results in this reaction. Soil extracting solutions (2M potassium chloride and 0.5M potassium sulphate) increased interferences by the organic compounds. A dialysis system was developed and its inclusion with the Technicon AutoAnalyzer II eliminated interferences caused by organic nitrogen compounds.

The results of the extraction study on the algal pond effluent revealed that ammonium, phosphate, and potassium can be measured in 0.1N hydrochloric acid extracts while nitrite and nitrate should be measured by direct filtration.

The results of the pot experiment conducted under greenhouse conditions indicated that the algal pond effluent had good effect on ryegrass yield but can not compete with the standard fertilizer application in terms

of ryegrass yield. There was a net release of nitrogen and phosphorus in the Darvel and Dreghorn soils due to mineralisation of waste solids and soil organic matter.

Four experiments were carried out in Pakistan for the evaluation of farmyard manure for its nitrogen, phosphorus and potassium contents. Farmyard manure produced by sheep was richer in nutrients than cattle manure. The nutrients are lost from the heaps of farmyard manure left uncovered and outdoors. There was variation in the nutrient level of fresh dung and urine excreted from individual animals but composite samples from a group of four buffaloes showed less day to day variability. The average chemical composition of farmyard manure would be the best estimate for the nutrient level if the fresh farmyard manure is to be applied to crops. The range of the farmyard manure production was from 23 to 43 kg per buffalo per night during winter while in summer it was from 13 to 25 kg per buffalo per night. Anaerobic storage increased the pH of farmyard manure while aerobic storage showed a decrease in pH. Soil or straw coverings reduced but did not prevent leaching losses of nitrogen and potassium. The plastic covering reduced leaching, the mineralisation of nitrogen and phosphorus but increased the gaseous losses of the nitrogen. The soil and straw coverings are, therefore, better than the plastic covering for the conservation of the nitrogen and phosphorus in the stored farmyard manure.

CHAPTER ONE

INTRODUCTION

The aim of this chapter is to provide a brief background to the work completed and presented in this thesis. The relevant research information and literature reviews are given in the corresponding chapters.

1.1. IMPORTANCE OF MAJOR PLANT NUTRIENTS

The role of major nutrients (N, P and K) in plant nutrition have been considered very important for the achievement of higher yield of crops. The requirement of crops for nitrogen is great. This nutrient is very complex in its behaviour. It occurs in soil, air and water in organic and inorganic forms. The main inorganic chemical forms of nitrogen in the soil-crop system are inert nitrogen gas, nitrous oxide, ammonia, ammonium, nitrite and nitrate but most of the soil nitrogen is in organic forms within the soil organic matter. The nitrogen is transformed from one form to the other. Nearly, all transformations of nitrogen are carried out by soil micro-organisms. Nitrogen can be mineralized due to decomposition of soil organic matter to produce ammonium-N which is then nitrified to nitrite and nitrate-N. Crop plants take up both ammonium and nitrate ions through their roots. Nitrate is reduced in the plants to ammonium, then to simple soluble organic molecules, amino acids,

amides and amines by enzymes within the plant. Nitrogen is very mobile within the plant.

Wild (1988) has summarized the beneficial and harmful effects of nitrogen on plant growth as follows:-

1. It increases leaf size and therefore the potential for greater photosynthesis, which will increase root growth, total dry matter and yield of useful product.

2. It increases tiller survival in cereals.

3. It increases the protein content of the storage organs that is grain, tubers or roots.

4. It increases the size of plant cells and reduces the thickness of their walls.

5. It increases the proportion of water in the plant fresh weight because of increased plant protoplasm.

6. It darkens the green colour of the leaves due to greater production of the chlorophyll.

7. It increases the shoot/root ratio.

If the addition of nitrogen is excessive, the harmful effects that can occur are:-

1. A greater susceptibility of the plant to attack by pests and diseases and to damage by drought and frosts, as a result of the thinner cell walls.

2. With cereals, the straw is weakened so that the crop is liable to lodge.

3. The quality of crops such as sugar-cane, sugar-beet and malting barley grown mainly for their carbohydrate is reduced.

4. Weed competition can become severe which will reduce crop growth.

5. Where water is in short supply, the greater transpiration loss from the increased leaf area may reduce final yields.

6. There is a delay in crop maturity.

Phosphorus is present in the soil as organic and inorganic forms. Organic forms of phosphorus are an essential part of the soil organic matter. Micro-organisms require phosphorus and any deficiency in soil phosphorus leads to less metabolism and activity. The organic forms are transformed into orthophosphate by enzymic action before being taken up by the plants. Several calcium phosphates occur in the soil which are slightly soluble. The inorganic soil phosphorus relevant to crop uptake is

held tightly in the soil in association with calcium, aluminium and ferric iron. These may occur as direct phosphate compounds or in association with hydroxides particularly of aluminium and iron. Phosphate is also held at the edges of the clay minerals in association with aluminium. Calcium carbonate can also adsorb soil phosphate.

Crop growth can become stunted due to the deficiency of phosphorus. Seeds have high phosphorus while its level in the straw is low. The main source of plant-available phosphorus is generally the labile pool. This provides fairly rapid exchange with the soil solution which maintains the solution concentration. In general, the labile pool can be considered as orthophosphate adsorbed on the surfaces of hydrous oxides and carbonates plus calcium, iron and aluminium phosphates. Archer (1988) states that the variation in soil phosphate supply to crops will depend on the concentration of phosphate in the soil solution and the buffer capacity or ability to replenish that solution from the labile pool. If the buffer capacity is high, a lower soil solution concentration may be adequate. Crops need an adequate phosphorus supply throughout their growing season, but particular importance has been attributed to adequate phosphate for young plants for their root development.

Potassium is considered as the second most important major nutrient next to nitrogen in crop nutrition. Potassium occurs in the soil solution as the simple cation. Crops take up potassium in its simple cationic

form. Potassium is not a component of proteins and carbohydrates. The absorbed potassium is mostly retained in the cell sap. It regulates the osmotic pressure and maintains the turgidity of the plant. Potassium plays an important role in photosynthesis and respiration as well as in the transference of the carbohydrates from one part of the plant to another. It is extremely mobile in the plant. Its luxury uptake can interfere with the uptake of other ions. For example, magnesium deficiency can occur in crops due to high potassium levels in the soil and its luxury uptake by the crop can lead to hypomagnesaemia in grazing animals. In the case of potassium deficiency, the plants transfer it from older to younger leaves in their efforts to survive. This transference could lead to the appearance of the potassium deficiency in the older leaves. Near the maturity of the crop when the leaf cells start to decay, potassium movement starts backward to the soil.

The amount of soil phosphorus and potassium available to a crop is the result of the balance between inputs (fertilizers, organic manures and soil reserves) and offtake (crop removal). MAFF (1985) stated that removal of phosphorus and potassium from the soil depends on the type of crop and the level of yields. Table 1.1 shows the removal of phosphate and potash by various crops.

Table 1.1 Phosphate and potash removal by crops(fresh material MAFF, 1985).

Crop	Phosphorus (kg/tonne)	Potassium (kg/tonne)
Cereal		
grain	3.1	4.5
straw	0.6	6.6
Oilseed rape - seed*	6.4	8.8
Dried peas*	3.5	8.0
Vining peas*	0.7	2.6
Field beans*	4.4	9.6
Potatoes - tubers*	0.4	4.6
Sugar-beet - roots	0.3	1.7
- tops	0.4	4.6
Kale	0.5	4.0
Maize - forage	0.4	2.9
Swedes - roots	0.3	1.9
Broad beans*	0.6	2.9
French beans*	0.4	1.9
Beetroot	0.4	3.6
Cabbage	0.4	2.9
Carrots	0.3	2.4
Cauliflower	0.6	3.1
Onions - bulb	0.3	1.4
Sprouts - buttons	1.0	5.0
- stems	0.8	5.8
Grass - silage	0.7	3.8
- hay	2.4	14.4

* Haulm not removed

Archer (1988) reported the uptake of major nutrients as shown in Table 1.2.

Table 1.2 Amounts of major nutrients removed in crops (Archer, 1988).

Crops	Percentage dry matter at harvest	N	P	K
		-kg/t fresh material-		
Cereals				
grain	85	17.0	3.4	4.7
straw	85	6.0	0.7	6.8
Sugar-beet				
roots	22	1.7	0.3	1.8
tops	16	3.2	0.5	4.8
Potatoes				
tubers	22	3.0	0.4	4.8
Oilseed rape	92	33.0	7.0	9.0
Grass				
silage	20	5.4	0.6	4.0
hay	85	14.0	2.6	15.0
Kale	15	3.6	0.5	4.2

Wild and Jones (1988) reported the amounts of major nutrients present in the crops as shown in Table 1.3.

Table 1.3 Approximate amounts of major nutrients present in the harvested parts of agricultural crops (Wild and Jones, 1988).

Crop	Amount of nutrients kg/tonne of dry matter N	P	K
Wheat - grain	20.0	4.0	5.5
- straw	7.0	0.8	8.0
Maize - grain	20.0	4.0	10.0
- straw	10.0	2.0	12.0
Rice - grain	18.0	4.0	5.0
- straw	4.0	1.0	10.0
Soy-beans	30.0	4.0	7.0
Sugar-beets - roots	7.5	1.5	8.0
- tops	20.0	3.0	30.0
Potatoes - tubers	14.0	2.0	22.0
Oilseed rape	36.0	7.0	10.0
Ryegrass hay	16.0	3.0	18.0
Kale	24.0	3.5	28.0

The variation in amounts of nitrogen, phosphorus and potassium removed by crops may be due to the differences in the amounts available in the soil at a specific time in the specific area, the factors responsible for restricting the nutrients availability (chemical, physical, biological and climatic), and types of crops and their yields.

1.2 SOURCES OF PLANT NUTRIENTS

There are several sources of the major elements in the environment, both natural and artificial such as weathered soil parent material (rocks), commercial fertilizers, organic manures, sewage sludge, irrigation waters, municipal refuse and waste waters,. Organic manures consist of either:-

1. Crop residues, left in the field after harvest.
2. Green manuring crops (mainly N-fixing legumes).
3. Organic wastes from agriculture (such as slurry and farmyard manure).
4. Organic wastes from processing agricultural produce (such as oil cakes and rice husks).
5. Other organic wastes (night-soil and household refuse).
6. Composts prepared from the above materials.

Biofertilizers so far proposed or used include:-

1. N-fixing organisms (Azolla and blue-green algae).
2. P-mobilising organisms (mycorrhizae).

1.3 USE OF ORGANIC MANURES AND NUTRIENTS LOSSES

Historically, organic manures have been important for maintaining soil fertility. However, in recent decades a ready supply of cheap inorganic fertilizers has dominated the use of organic manures.

Estimates of livestock waste production on UK farms based on June 1990 census figures (MAFF, 1990) suggest a total annual output of about 191 million tonnes, of this approximately 78 million tonnes was slurry or manure

collected from buildings and yards and requiring handling and storage. Manure utilization may be poor because management practices lead to nitrate leaching, ammonia volatilization and denitrification losses. Chalmers et al. (1992) reported that farmers make small, inconsistent decreases in inorganic fertilizer rates applied to crops following applications of organic manures. Even a modest improvement in allowances for nutrients supplied from organic manures could result in major savings in fertilizer costs without loss of yield and with less environmental pollution. Smith and Chambers (1993) stated that organic manures contain valuable quantities of nitrogen, phosphorus and potassium, but many farmers regard them as waste material rather than as source of plant nutrients.

For the efficient use of the nitrogen in organic manures application rates and timing must be matched to crop demand under the climatic conditions. It means that storage is generally necessary over the autumn and winter period. However, storage facilities are often inadequate because large quantities of wastes are produced and storage installations are expensive. Nitrogen losses by leaching, ammonia volatilization and denitrification can be substantial, depending on weather factors and soil conditions. Lousier and Parkinson (1978) reported that leaching can remove significant quantities of inorganic ions. Potassium is often the most readily lost ion. Germon et al. (1979) reported that mineralisation of the organic matter in pig slurry is very slow which means that much of

it is not available to the crop in the short term, though it contributes to the nutritional pool in the soil. Phosphorus is present in pig slurry in soluble inorganic forms and as organic compounds which can mineralize in soil. Soluble forms are scarcely mobile in the soil. Their distribution within soil profile depends on soil characteristics such as pH and organic matter. According to Vetter and Steffens (1981), 8 to 13% of phosphorus in pig slurry can infiltrate down the soil profile, reaching 90 cm depth in acid soils. MAFF(1982) described that if chicken farmyard manure is stacked in the open, considerable nitrogen and potassium can be lost by leaching of rainwater through the heaps. Some seepage losses of liquid from the heaps may also occur. Gaseous loss of ammonia may take place when the heap is uncovered. Unwin (1986) reported that high rates of poultry manure (15-45 t/ha annually for 6 years) and pig slurry (100 and 200 m^3 in 1983) applied in autumn to sandy arable soils led to overwinter nitrate leaching losses of equivalent to over 400 and over 300 kg N/ha, respectively. By contrast, the annual leaching losses from grassland and chalk soil were equivalent to approximately 40 kg N/ha after autumn cattle slurry applications supplying approximately 500 kg N/ha. Thompson et al.(1987) reported that injection of slurry into grassland decreased ammonia volatilization to 2% of the ammonium nitrogen applied, compared with losses from surface application in autumn and spring of 74% and 48%, respectively. Archer (1988) reported that upto 20% of the total nitrogen may be lost after a few month's storage

of slurry. Pain and Thompson (1989) reported that after pig slurry application the ammonia volatilization losses rose from 9 to 78%. Injection of slurry and dilution can reduce ammonia volatilization losses of applied nitrogen. Borggaard et al. (1990) reported that organic matter affected phosphate adsorption indirectly by inhibiting aluminium oxide crystallization. The resulting poorly crystalline oxides had high phosphate adsorption capacity. In contrast, the influence of organic matter on the crystallinity of the iron oxides, and therefore on their capacity to adsorb phosphate, seemed limited. Jarvis and Pain (1990) stated that rapid rates of ammonia loss can occur during the first few hours after organic manure application to land. With slurries, approximately 40 to 50% of the total loss often occurs within 6 hours, 70% within 24 hours and more than 90% over 5 days. A wide range of ammonia volatilization losses has been reported. Frost et al. (1990) reported ammonia volatilization losses from the applied cattle slurry ranged from 7 to 84%. Similarly, Unwin et al. (1991) concluded that when organic manures containing abundant available nitrogen for example, cattle or pig slurry or poultry manure are applied to freely draining arable or grassland soils in the period September to December, considerable nitrate leaching losses are likely to occur. Sommer and Olsen (1991) suggested that dilution of slurry can decrease ammonia volatilization losses. They also reported that surface application of cattle slurry to grassland in December resulted in a loss of 30 kg N/ha (equivalent to

12% of slurry N applied) compared with 4.5 kg N/ha (equivalent to 2% of slurry applied) from an April application. Bernal et al.(1993) studied the nutrient balances in calcareous soils after application of different rates of pig slurry. They reported that total N and exchangeable K increased after slurry applications of 300 m^3 /ha or more, and available P increased after the smallest application rate (100 m^3/ha). Maximum crop nutrient uptake of 41, 40 and 91% for N, P and K occurred with the smallest dose of slurry. Large losses of N, ranging from 27 to 74% (mean 55%) of N added to soil, occurred with all slurry treatments. From 41 to 71% (mean 55%) of the total P added in pig slurry was fixed in non-assimilable forms. Most of the K from the pig slurry was available to the plants. Zhu and Xi (1989) reported that the availability of inorganic P in manures is high, while that of organic P depends on its mineralisation rate. In field situations, crops recovered 12 to 18% P from farmyard manure in first year. Addition of P as inorganic fertilizers will be necessary to maintain soil fertility. Munoz et al. (1990) reported that four chicken manure samples (two fresh, one 2 months old and the other 24 months old) were evaluated for pH, total and available N, P and K contents. Total N ranged from 2.47 to 3.72%, the lowest value corresponding to the 24-month old manure. There was little variation among manures as to total P and K, averaging 18,588 and 24,238 mg/kg, respectively. All the K of chicken manure was in the exchangeable form. Available phosphorus increased with age of the manure.

This P fraction represented 10, 7, 68 and 97% of total P for fresh (1), fresh (2), 2-month old and 24-month old manures, respectively.

1.4 EFFECTS OF MANURE ON SOILS AND CROPS

Manure application to soils increases soil fertility and improves quality of the crops. Simpson (1986) pointed out that manures had two main functions - to supply nutrients and to supply organic matter. He described that the concentration of nutrients in manures is very low compared with that in fertilizers. One tonne of traditional farmyard manure made with straw supplies nitrogen, phosphorus and potassium in similar amounts to 50 to 100 kg of a modern concentrated compound fertilizer. However, manures also supply useful amounts of calcium, magnesium, sulphur and trace elements, all largely neglected in modern fertilizers. If well conserved and incorporated into the soil they can give considerable savings in the amounts of fertilizer required. Appreciable proportions of the total nutrient content of manures occur in complex organic forms which have to be mineralized before they release available nutrients. Thus, not all will be available for the first crop after application. Organic matter is attacked and transformed by micro-organisms when returned to the soil. Much of the carbon is converted to carbon dioxide and makes no long-term contribution to the organic matter content of the soil. Other parts of the organic matter are converted to humus, a black or dark brown, colloidal, very complex organic

material which remains in the soil. All manures make some contribution to long-term soil fertility and the maintenance of humus in the soil. In fact, very large amounts of manures need to be applied to have significant long-term effects on soil organic matter content of the soil. It is mainly due to the high water content of manures and slurries. Thus, the use of bulky straw-based farmyard manures can be expected to make only a small but useful contribution to the quantity of humus in the soil. Slurries will contribute less. The fate of the farmyard manure could be as follows:-

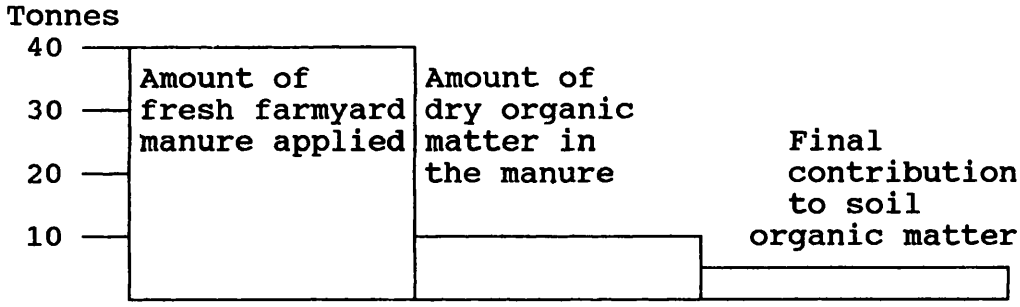

Figure 1.1 Fate of farmyard manure in soil (Simpson, 1986)

Magdoff (1978) reported that soils that have high rates of mineralisation of soil organic matter may also mineralize organic matter from manure relatively rapidly. This may have a beneficial effect for crops growing on medium to coarse texture soils that are well drained. Organic nitrogen in manure applied to these soils may be quickly released in an available form for plants. However, it also means that more manure has to be applied to these soils in order to maintain their organic matter levels

when under continuous cultivation. Zane and Basil (1980) reported that land spreading of dairy manure is effective both in disposing of waste and in utilizing plant nutrients in the manure. They concluded from their study that high rates of manure increased soil pH and soil contents of potassium, magnesium and phosphorus and also increased the cation exchange capacity of soils. These factors contribute toward higher crops yield on manured soils. Klausner and Guest (1981) reported that dairy manure contains an appreciable amount of ammonia nitrogen and if left exposed on the soil surface, it is easily lost by volatilization. Two methods of row applied liquid manure were made on corn (Zea mays L.) during the early stages of the growing season to identify the yield response to ammonia conservation. The use of manure significantly increased yield in comparison to no manure. Sommerfieldt and Chang (1985) reported that application of feedlot manure increased the organic matter contents of both irrigated and nonirrigated soils. Sommerfieldt et al. (1988) noted that long-term application of cattle feedlot manure (C/N ratio of ca. 10.2) application lowered the C/N ratio of soil (ca. 8.2) by small amounts. Ndayegamiye and Cote (1989) studied the effect of long-term pig slurry and solid cattle manure application on soil chemical and biological properties. The soil under study was an acidic silty loam soil. They reported that neither treatment affected soil pH, total N and C/N ratio compared to the control. The cation exchange capacity of the soil was significantly higher with cattle manure treatment than

with control or slurry. The highest rates of cattle manure also increased soil organic carbon, microbial activity and potentially mineralized nitrogen. The soil microflora populations (bacteria, fungi, actinomycetes, ammonifiers and nitrifiers) were greatly improved by both treatments. Whitehead et al. (1989) reported that incorporation of the slurry increased the yield of ryegrass, the concentration of nitrogen in the herbage and nitrogen uptake. William (1992) applied cattle manure to corn. He stated that corn yield and nitrogen uptake was increased with manure. Application of manure resulted in similar or slightly lower soil profile nitrate than agronomical equivalent rates of fertilizer nitrogen.

1.5 NUTRIENT RECYCLING FROM MANURES

The two main organic manures applied to agricultural land are animal wastes and sewage sludges. Application of these manures provides an effective means of disposal of these materials. It also allows the recycling of organic matter and nutrients back to soil. A constraint on disposal of the farm animal wastes to the land is the risk of pollution of streams or ground water if very high rates are applied at inappropriate times. Leaching through the soil can occur or in extreme cases direct run-off of manures into ditches or streams may take place. Odours are a problem in some situations. Daily application of the fresh manure or slurry looks impracticable. Manure is stored on the farm for several months.

Aerobic and anaerobic treatment of manure or slurry reduce the biological oxygen demand (BOD) thus controlling odour. Grundey (1980) described that the fundamentals of aeration are that a population of oxygen consuming micro-organisms is developed and they feed on the organic waste for growth. The products are carbon dioxide, water, heat and dead micro-organisms or sludge, and commonly 0.5 to 0.8 kg of sludge may be produced for every kilogram of BOD removed. Composting of a solid manure is a process whereby a loose textured heap of manure, usually mixed with straw, is kept damp and aerobic digestion takes place. The material is slowly converted to a friable, damp, stable, non-smelling organic mass. The anaerobic digestion process is a dynamically mixed one comprising three stages: (1) polysaccharides in the waste are hydrolysed to simple sugars; (2) these are then converted into hydrogen gas and carbon dioxide to give some methane plus some acetic acid; and (3) methane bacteria convert acetic acid to more methane. Whether aerated or non-aerated, treated wastes, although they are free of smell can still be considered as pollutants for the environment and ground waters.

Liquid manures and slurries may be treated either aerobically or anaerobically before their disposal. The solid and liquid fractions of the slurries may also be separated. The liquid fraction still contains high levels of nitrogen and phosphorus. With such effluents, there is a potential for the use of algal cultures to treat the waste waters and production of animal feeds. The main purpose of the conversion of inorganic nutrients of animal

waste to algal biomass is to stabilize the nutrients in an organic form. Although the direct use of algae as an animal feed would be limited by its high moisture content and the high cost of conventional drying, sun drying or mixing with dry feeds are possibilities. In the case of swine and hog production, a wet feed is feasible. The high rate algal pond (a shallow mixed raceway type pond) first proposed by Oswald (1963) for waste waters treatment maximizes both waste waters treatment and algal production. During photosynthesis the algae produce oxygen, it is this oxygen which keeps the system aerated and so maintains the aerobic conditions required by the bacteria. Martin and Fallowfield (1989) stated that algal photosynthesis provides a potential oxygen source to meet the biological oxygen demand (BOD) of wastewaters. The organic material entering the pond is being oxidatively degraded by the heterotrophic bacteria in the pond solution. The degradation produces carbon dioxide, ammonium, nitrite, nitrate and phosphate ions which are released into the pond solution. Ammonium, nitrite, nitrate and phosphate ions are taken up by the algae, and immobilized in algal biomass. Fallowfield et al. (1992) reported that the high-rate algal pond is an efficient treatment system for controlling wastewater pollution by reducing the organic matter and the inorganic nutrient content. Ben-Amotz and Avron (1989) reported that algae accumulate surplus phosphate in their cells. Bental et al. (1988) determined that most of the intracellular phosphate was in the form of polyphosphates. The algal

biomass can be used for fuel production and as fertilizer. However, the high cost of algal removal and the disposal costs of the algal sludge generated are two major limitations.

The effluent along with algal biomass can be applied to the agricultural lands as a green irrigation. If applied to the land, the mineralisation of the algal dry matter would be slow and the pollution danger by direct application of slurries with high level of nitrogen and phosphorus can be minimized. In fact, it seems difficult to ensure that algal pond effluent would be free of inorganic nitrogen and phosphorus. The need for the evaluation of the algal pond effluent remains. Studies on the application of these effluents to crops will throw light on the mineralisation rate of the algal dry matter in the soil, their effects on crops in term of yields and nutrient balance in the soil.

1.6 USE OF MANURES IN DEVELOPING COUNTRIES

Organic manures are valuable inputs due to their positive effects on improving the soil fertility and crops yields, but farmers of developing countries are not taking benefits. Several reasons can be given for less use of organic manures such as fuel shortage, electricity load shedding, lack of awareness about manure benefits as fertilizer based on scientific knowledge. Schoningh and Wichmann (1990) noted that Indonesian farmers found it difficult and uneconomical to collect cow dung from scattered locations on which the cattle grazed. They

estimated that in 1986, 54,000 tonnes N, 215,000 P, and 178,000 tonnes K were potentially available from cow and buffalo excreta. However, from these amounts, only 6,650 tonnes N, 1,635 tonnes P and 3,000 tonnes K originating from dairy cattle were utilized as manure. Similarly, in Pakistan, only about half of the available animal droppings were collected, of which some 50% was estimated to have been used as organic manures, the rest being burnt as dung-cake fuel. In the Philippines, the common practice among farmers was to burn the crop residues, and to dispose of animal manures directly to the environment. The only exception was chicken manure, which was sold to vegetable growers and to fish farm operators. Farmers in Sri Lanka found that cattle dung containing weed seed from the soils in the lowlands grazing areas can spread weeds.

Schoningh and Whichmann (1990) showed the fate of nitrogen from farmyard manure (Figure, 1.2).

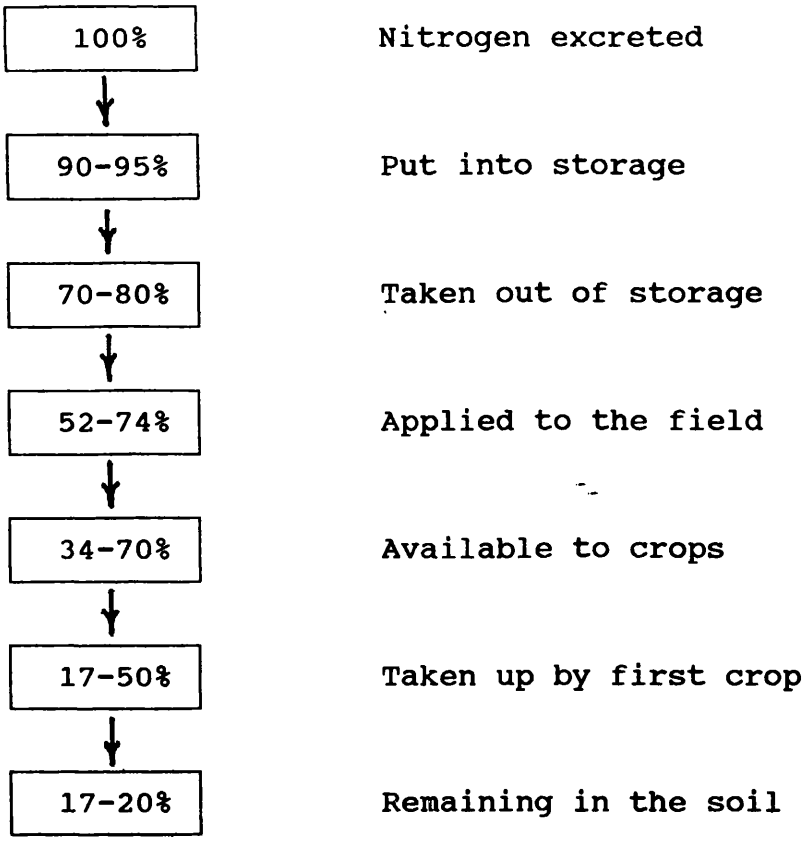

Figure 1.2 Fate of nitrogen from farmyard manure

They also advocated that there will always be deficits in the nutrient balances of the developing countries due to the following factors:-

1. Substantial amounts of food are necessary to supply the urban population, which today already accounts for approximately 40% of the total population in developing countries.

2. The export of nutrients with agricultural produce, such as coffee and soya sold in the international market.

3. The unavoidable losses (especially of N) that occur during storage, transport and application of organic manures: these deficits will have to be compensated by the use of mineral fertilizers.

Finally they concluded from their study that there are several positive effects which make organic manures a valuable input to improve soil fertility. Organic material promotes biological activity, nutrient exchange capacity, water holding capacity, organic matter content and the structure of the soil. A better nutrient retention of the soil and better root development of the crop which are achieved by the organic manure application finally helps to improve the efficiency of mineral fertilizers on crop yield, thus making their use more economic.

Schoningh and Whichmann (1990) reported that 50% manure is applied to the land in Pakistan, this figure seems an overestimate in the light of personal experience. In Dera Ismail Khan, use of manure as fertilizer material will be hardly 5% in irrigated areas while it is not applied to the land in rainfed areas at all. Only the excreta of grazing animals goes directly to the land. In the near future, the increased use of the farmyard manure looks possible because of the start of water supply through a newly built Chasma right bank canal in Dera Ismail Khan area.

1.7 DETERMINATION OF AMMONIUM IN SOIL

Accurate determination of nitrogen in soil extracts and acid digests is an important consideration for

fertilizer recommendations and to know the contributions of soil and applied nitrogen towards soil fertility, pollution problems and crop response. Accuracy in nitrogen determination by any method is more important in studies where nitrogen dynamics are critical to the interpretation of results.

Ammonium in soil can be measured by manual as well as by automated methods. The usual manual method followed by most researchers is steam distillation (Bremner, 1965). An automated version of the Berthelot colour reaction for ammonium determination in soil extracts and Kjeldhal digests is also widely used. Searle (1984) described that the Berthelot reaction is the name given to the reaction of ammonium ions and a phenol, which under suitable oxidising conditions, result in the formation of an indophenol dye. Indophenol dyes are highly conjugated and absorb strongly between 630 and 720 nm. Methods based on this reaction are sensitive and relatively specific for the ammonium ion. There are numerous methods in which ammonium nitrogen is determined by this reaction and this also applies to total nitrogen methods in which nitrogen is converted into the ammonium form by suitable pre-treatment such as Kjeldahl digestion. Automated versions of the Berthelot reaction are widely used where large number of samples require nitrogen determination. The automated reaction has also proved popular because problems caused by the complex reaction equilibria are effectively eliminated in systems where reaction conditions are closely controlled. Automated methods are

adaptations of manual methods and are subject to the same reaction variables such as pH, reagent concentration, sequence of reagents, colour development time and temperature and interferences by organic nitrogen compounds.

White and Gosz (1981) compared an automated Berthelot method for determination of inorganic ammonium-N with the distillation method for analysis of potassium chloride extracts of forest floor samples. They reported that amino acids added to the potassium chloride extracts were found to contribute significant positive interference to the automated method. Modification of the automated method yielded results identical to steam distillation analyses. The original method was found to overestimate inorganic ammonium-N in extracts of forest floor material by 17 to 26 percent. Burton et al.(1989) concluded that small but significant errors in the estimation of the ammonium content of soils may result from direct automated determination of ammonium content of soil extracts using the automated indophenol procedure (Technicon Industrial Method No. 98-70W). Amino acid interference may be particularly important in soil samples, and in studies of the inter-conversion of organic and inorganic forms of nitrogen. Searle (1990) found that positive interferences by amino acids in the determination of ammonium in soil extracts by automated indophenol methods can be minimized by using the nitroprusside catalysed reaction. Hydrolysis of amino acids is virtually eliminated because this

reaction enables the use of low sample volumes, reagent concentration and reaction temperatures.

Ammonium can be measured directly in salt extracts of soils and in Kjeldahl digests. In methods that are applied directly to sample digests the possibility of interferences can be minimised by high sample dilution and the use of a complexing agent. Under these conditions the nitroprusside-catalysed reaction is nearly always used because the increased sensitivity offsets the dilution factor. Other methods of overcoming interferences are the use of flow dialysis and distillation. The use of automatic distillation or dialysis, however, does increase the complexity of the flow system and this usually decreases the rate of sample analysis.

1.8 AIMS OF THESIS

The major nutrients required by crops are nitrogen, phosphorus and potassium. Manures and inorganic fertilizers are applied to maintain the soil fertility and higher yield of crops. Disposal of farm animal wastes to agricultural lands may cause pollution problems by leaching, seepage through manures heaps and ammonia volatilization. There is need to explore alternative ways of waste disposal safely to the environment. Shortage of commercial fertilizers in the developing countries necessitates the evaluation of the manures for their potential to supply nutrients. For the efficient use of manures, conservation of these nutrients seems essential through improved storage.

The work presented in this thesis can be divided into two parts. The first part was completed in this Department while the second part concerned with the farmyard manure was completed at the Faculty of Agriculture, Gomal University, Dera Ismail Khan, Pakistan.

The objectives of the work completed at Glasgow were:-

1. Analytical studies were carried out for the determination of ammonium-N in salt solutions and attempts were made to improve the method of ammonium-N determination based on the Berthelot colour reaction by the elimination of the interferences caused by organic compounds. As the result of the method development, a gas dialysis step was included in the ammonium-N main manifold of the Technicon AutoAnalyzer II.

2. High-Rate Algal Ponds were being run by Dr.Fallowfield at the Scottish Agricultural College, Auchincruive, Ayr. The algal pond effluent was evaluated by carrying out the following studies:-

(A) Chemical extraction involving four extractants was performed for the selection of a suitable extractant for determining the available nutrients in the algal pond effluent.

(B) Algal pond effluent was evaluated for its potential to supply nutrients to ryegrass by conducting a pot experiment under the greenhouse conditions. The

nitrogen balance was also investigated in this study to evaluate the algal pond effluent for its potential for pollution through its nutrient content if applied as organic irrigation to crops.

The second part of the work presented in this thesis was completed in Pakistan by conducting the following studies:-

(A) A small survey of farmyard manure heaps was made in the village Lundah Sharif located 15 miles to the south of Dera Ismail Khan to determine the nitrogen, phosphorus and potassium contents of the manure.

(B) A study was undertaken to investigate the nutrient levels in the fresh excreta of a group of four buffaloes, manure production with and without sawdust bedding and nutrient levels in it.

(C) Manure produced from buffaloes was stored by different methods with the aim of finding out an economical way for manure storage to achieve the goal of the nutrient conservation.

CHAPTER TWO

METHODS

This chapter is intended to describe the routine analytical techniques used in this study.

2.1 WASHING OF GLASSWARE

The shaking bottles, bottle tops, filter funnels, beakers, stirring rods and volumetric flasks were first washed with hot water and soaked overnight in a 2% solution of Decon 90 (Decon Laboratories limited). These were then washed with hot water, rinsed twice with deionized water and finally dried in an oven at 70°C.

2.2 WASHING OF FILTER PAPERS

Filter papers used throughout the study were washed before their use. Each filter paper was folded in a clean and dry plastic funnel. It was washed with 50 ml of 0.5M sulphuric acid by filtration in equal portions of 25 ml each. Then these were rinsed several times with deionized water to wash any acid deposit. Universal pH paper was used as a check to be certain that filter papers were acid free. The washed filter papers in the funnels were dried for four hours in the oven at 70°C (Shah,1988).

2.3 DETERMINATION OF SOIL pH

Soil pH was determined in a 2.5:1 water:soil mixture by a combined glass-reference electrode and pH meter. The pH meter was standardised with buffer solutions of pH 7.0 and pH 4.0. The buffer solutions were prepared by dissolving buffer tablets in 100 ml deionized water.

Duplicate 12 g sample of each soil were weighed into 2 oz glass bottles and 30 ml deionized water was added to each bottle. The suspension was shaken for half an hour on an end-over-end shaker. The electrode was then immersed in the bottle and the soil suspension stirred by swirling the electrode slightly. The pH was recorded immediately.

2.4 DETERMINATION OF SOIL MOISTURE

The washed basins were dried in the oven at 110°C for an hour, cooled in a desiccator and weighed. A suitable weight of fresh soil was placed in each basin. The basins with soils were transferred into an oven for 24 hours at 110°C. Then basins were taken out of the oven and cooled in a desiccator and reweighed. The % moisture content was determined on an oven dry basis as follows:-

$$\% \text{ Moisture} = \frac{\text{weight fresh soil - weight oven dry soil}}{\text{weight oven dry soil}} \times 100$$

2.5 DETERMINATION OF MACRONUTRIENTS IONS

The Technicon AutoAnalyzer II was used for the analysis of ammonium-N, nitrite-N, nitrate-N and phosphate-P because of its sensitivity, speed and ease of use. The Technicon AutoAnalyzer II system consisted of sampler, proportioning pump, a water bath with constant temperature and colorimeter equipped with either 530 or 650 or 880 nm filters and phototubes. Results of the samples were recorded with a single pen chart recorder. The system was connected to a BBC microcomputer which was used for the measurement of peak heights and calculation of results. The reagents bottles were also put in a separate water bath with a constant temperature of 25°C.

The Corning EEL flame photometer was used to determine the potassium in soil extracts and acid digests.

2.5.1 Determination of Ammonium-Nitrogen

Ammonium nitrogen was measured by a modification of the indophenol green method using a complexing reagent to prevent interferences due to the precipitation of hydroxides in the reagent system. With the inclusion of a sodium nitroprusside catalyst, the sensitivity of the method was such that ammonium could be determined in the range of 0 to 1 ppm and with care 0 to 0.1 ppm (Brown, 1973). This method is applicable to water samples and a wide range of soil extractant solutions acid digests of plant or soil material. The schematic diagram of the flow system is shown in Figure 2.1.

2.5.1.1 Reagents

Analar grade reagents and deionized water were used throughout.

Alkaline phenol

22.5 g of sodium hydroxide was dissolved in about 800 ml deionized water in a 1 litre dark glass bottle and the resulting solution was degassed. 50 g phenol was weighed in a 1 litre beaker and approximately 600 ml sodium hydroxide solution was added and stirred with a glass rod to dissolve the phenol. The solution was returned to the bottle and the volume was made to 1 litre with degassed water and mixed gently.

Complexing reagent

50 g potassium sodium tartrate and 50 g sodium citrate were dissolved in 800 ml deionized water and degassed. 1.2 g sodium nitroprusside was weighed in a 100 ml beaker. 50 ml of degassed water was added to the beaker and stirred gently with a magnetic stirrer. The resulting solution was added to the citrate-tartrate solution. 0.5 ml of 30% Brij-35 was added and volume was made to 1 litre. The solution was then mixed gently.

Sodium hypochlorite solution (0.5%)

50 ml sodium hypochlorite solution (12% w/v available chlorine) was diluted to 1 litre with degassed deionized water and mixed gently.

Ammonium-N standard stock solution (1000 mg l^{-1})

Ammonium sulphate was dried for an hour at 110°C in the oven and cooled in a desiccator. 4.718 g dried ammonium sulphate was dissolved in deionized water and the volume was made to 1 litre. The solution was stored at 2°C. Working standards were prepared by dilution in the appropriate extracting solutions.

2.5.1.2 Procedure

The filtered solutions were analysed using the manifold shown in the Figure 2.1 alongwith standard solutions, blanks and zeros. The samples were run at the rate of 40 per hour and the colour was developed in the water bath at 37°C. The colour intensity was measured at 650 nm. The calibration graph for ammonium is linear from 0 to 5 mg NH_4-N l^{-1}. Samples with ammonium-nitrogen concentrations higher than 5 mg l^{-1} were diluted into the range 0 to 5 mg l^{-1} using an inbuilt diluter.

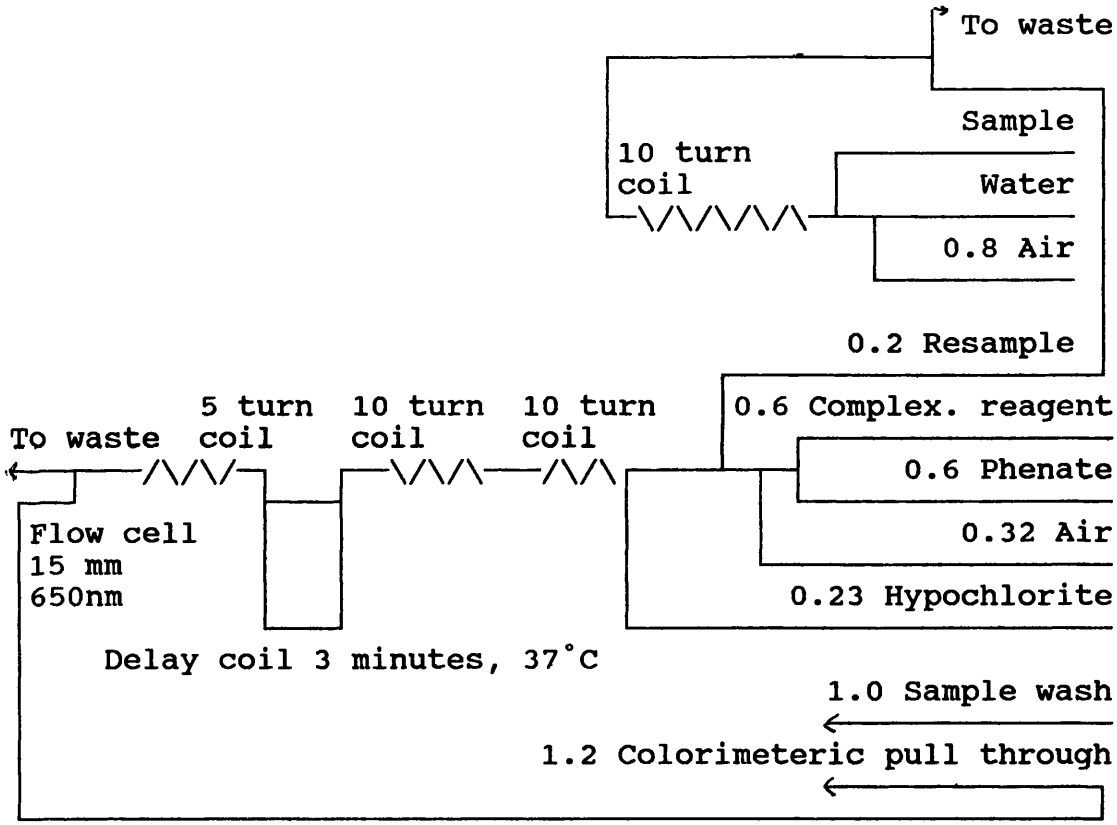

Figure 2.1 AutoAnalyzer Manifold For Determining NH_4-N

2.5.2 Determination of Nitrate and Nitrite-Nitrogen

In the automated system, nitrate nitrogen was reduced by hydrazine sulphate and copper sulphate to nitrite nitrogen followed by determination of the nitrite using the Greiss reagent. The method, therefore, measured nitrate plus nitrite. The nitrite nitrogen was measured separately on the same manifold by omitting the reduction reagents. The schematic diagram of the flow system for nitrate and nitrite is shown in Figure 2.2 (Best, 1976).

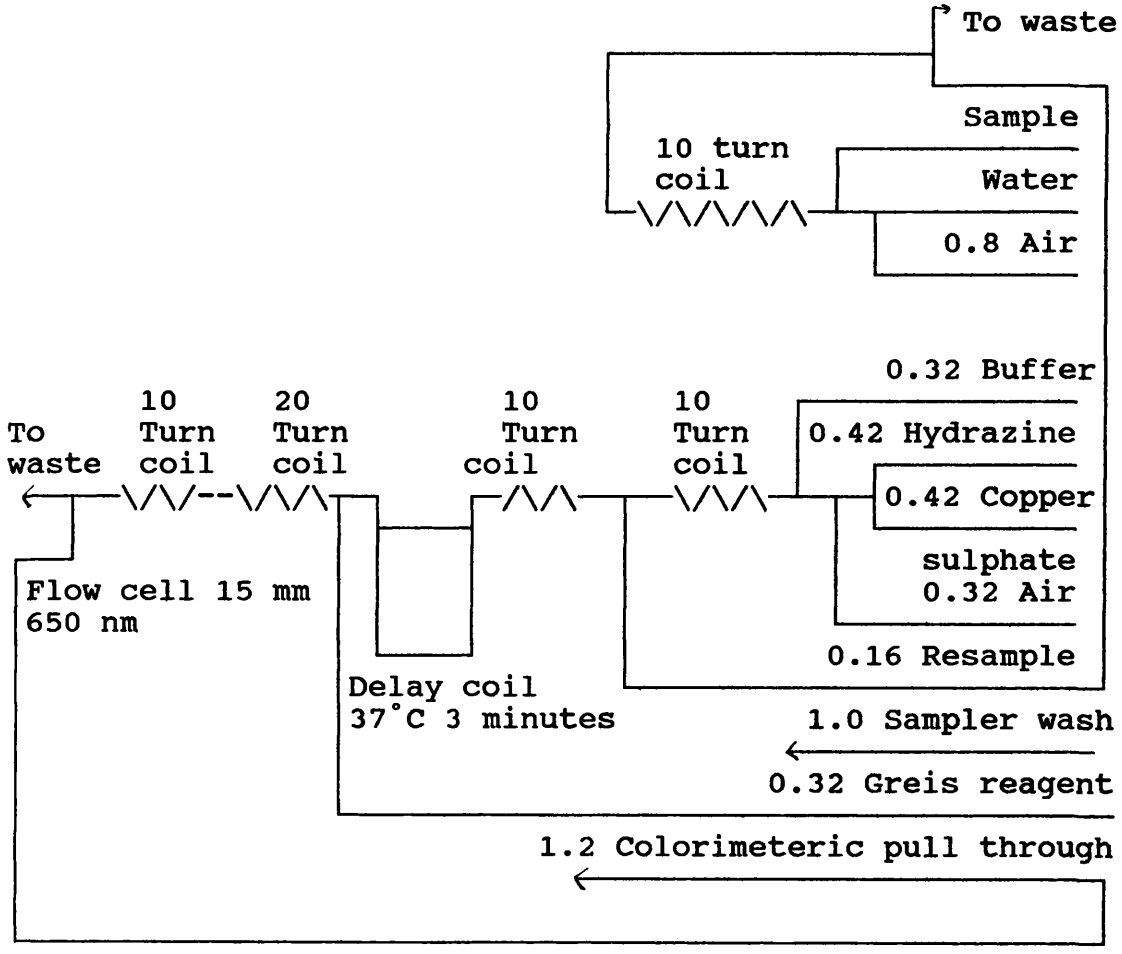

Figure 2.2 AutoAnalyzer Manifold For NO_2-N and NO_3-N

2.5.2.1 Reagents

Analar grade reagents and nitrogen free deionized water were used throughout.

Buffer solution.

22.5 g sodium tetraborate and 2.5 g sodium hydroxide were dissolved in 900 ml deionized water and the volume was made to 1 litre. The solution was then degassed.

Greiss reagent.

100 ml of concentrated hydrochloric acid was added into approximately 800 ml of deionized water and the solution was degassed. 10.0 g sulphanilamide and 0.5 g N-1-naphthylene diamine dihydrochloride were taken into a litre beaker. 500 ml of the acid solution was added to the beaker and stirred gently using a magnetic stirrer. This solution was returned back to the bottle, and the volume was made to 1 litre with degassed deionized water and mixed gently. This solution was stored in the cold room at 2°C.

Reducing reagent.

The reducing reagent was divided into two components.

1. Hydrazine sulphate.

0.30 g of hydrazine sulphate was weighed into a small beaker and transferred to a 1 litre volumetric flask containing approximately 900 ml degassed water. The volume

was made to the mark without shaking and hydrazine sulphate was dissolved by stirring with a magnetic stirrer keeping the top of the flask closed in order to prevent entry of oxygen. 0.5 ml of Brij-35 solution was added and the solution was mixed gently.

2. Catalyst solution.

1 ml of 2.47% copper sulphate solution and 0.5 ml of 30% Brij-35 solution were added to 1 litre of degassed water and mixed gently.

Nitrate-N standard stock solution (1000 mg l^{-1}).

Potassium nitrate was dried for one hour at 110°C and cooled in a desiccator. 7.222 g of dried potassium nitrate was dissolved in deionized water and the volume was made to 1 litre. The stock solution was stored at 2°C. Working standard solutions were prepared by dilution in the appropriate extracting solutions.

Nitrite-N standard stock solution (1000 mg l^{-1}).

Sodium nitrite was dried for one hour at 110°C and cooled in a desiccator. 4.928 g of dried sodium nitrite was dissolved in deionized water and the volume made to 1 litre. The solution was stored at 2°C. Working standard solutions were prepared by dilution in the appropriate extracting solutions.

2.5.2.2 Procedure

The filtered solutions were analysed for nitrate and nitrite using the manifold shown in the Figure 2.2 alongwith standard solutions, blanks and zeros. The samples were run at the rate of 40 per hour. The nitrate-nitrogen was reduced to nitrite by adding copper sulphate and hydrazine sulphate solutions to the sample as it passed through the water bath set at 37°C. The nitrite-nitrogen was determined by a diazotization coupling reaction whereby a pink colour was formed. The intensity of this colour was measured at 530 nm. Nitrate has a curved calibration in the range 0-5 mg NO_3-N l^{-1}. Samples having nitrate-nitrogen more than 5 mg l^{-1} were diluted into the range 0-5 mg l^{-1} using an inbuilt diluter.

For the determination of nitrite-nitrogen, the reducing reagents were replaced with nitrogen free deionized water containing 0.5 ml per litre of 30% Brij-35 solution. Nitrite has a linear calibration in the range of 0-1 mg NO_2-N l^{-1}.

2.5.3 Determination of Phosphate

Phosphate was measured using the Technicon AutoAnalyzer II. The method is based on the formation of a phospho-molybdate complex which is reduced using ascorbic acid to give a blue colour which may be measured at 660 or 880 nm. In order to speed up the formation of the complex, a small amount of antimony is added. The intensity of the blue colour is proportional to the phosphorous concentration in the original solution. The method is applicable to water samples and a wide range of soil extractant solutions and acid digests of plant or soil material. The schematic diagram of the flow system is shown in Figure 2.3.

2.5.3.1 Reagents.

Acid ammonium molybdate.

60 ml concentrated sulphuric acid was added to 800 ml deionized water in the fume cupboard and cooled. 5.2 g ammonium molybdate was dissolved in the dilute acid solution. 0.1 g antimony potassium tartrate was dissolved in 100 ml of deionized water in a beaker. This solution then was added to the dilute acid ammonium molybdate solution by stirring with a glass rod to avoid precipitation. The volume was made to 1 litre with degassed deionized water. The solution was stored in a dark glass bottle.

Ascorbic acid solution.

0.75 g ascorbic acid was dissolved in 100 ml of degassed deionized water.

Wetting agent solution.

0.5 ml Aerosol 22 was diluted to 1 litre with degassed deionized water.

Phosphate standard stock solution (1000 mg l^{-1})

Potassium dihydrogen phosphate was dried in an oven at 110°C for one hour. 4.3937 g dried potassium dihydrogen phosphate was dissolved in deionized water in a beaker. The content of the beaker was transferred to 1 litre volumetric flask and diluted to the mark with deionized water.

Working standard solutions were prepared by dilution in the appropriate extracting solutions.

2.5.3.2 Procedure

The filtrates were analysed for phosphate using the manifold shown in the Figure 2.3 alongwith standard solutions, blanks and zeros. The samples were run at the rate of 40 per hour. The colour was developed by ascorbic acid in the water bath at 37°C. The intensity of the colour was measured at 880 nm. The phosphate calibration graph is linear in the range of 0-5 mg PO_4-P l^{-1}. The samples having phosphate concentration higher than 5 mgl^{-1} were diluted by an inbuilt dilution system.

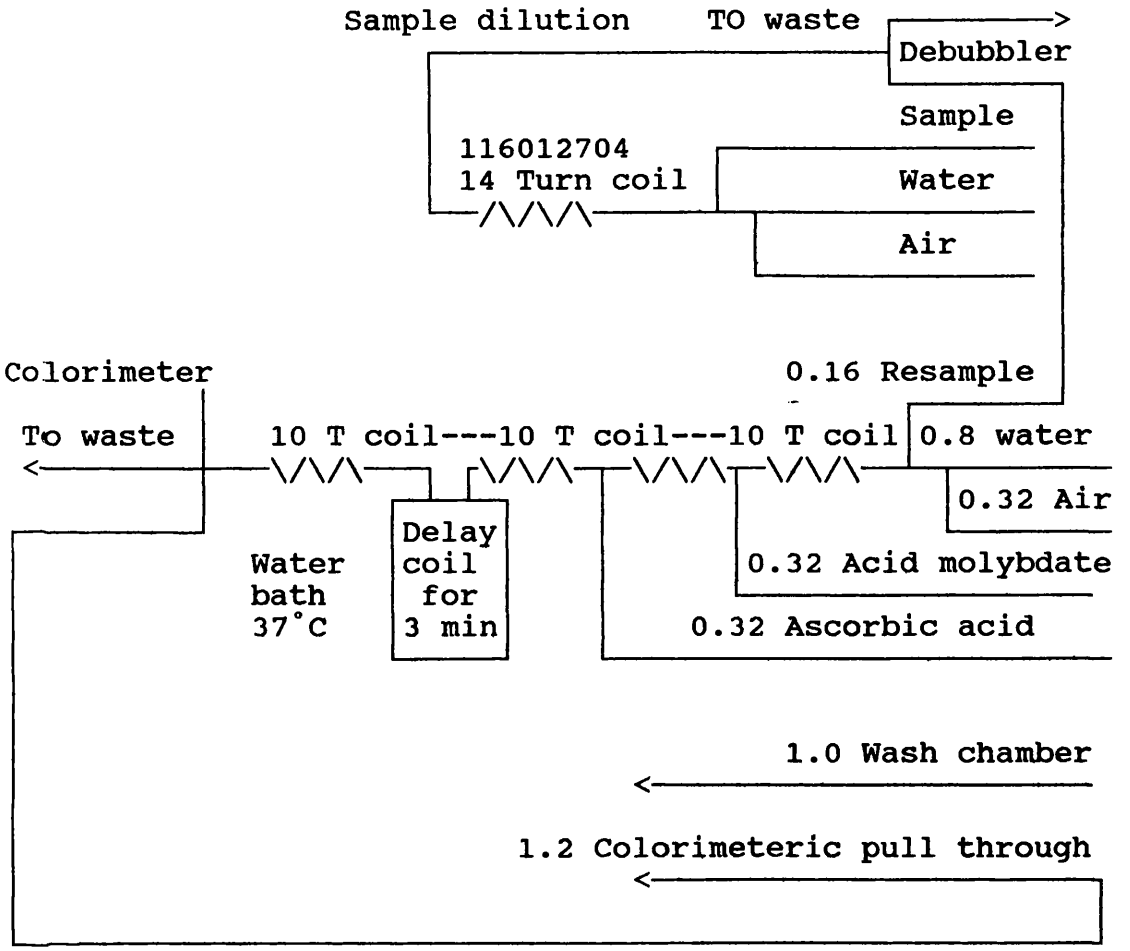

Figure 2.3 Technicon AutoAnalyzer II phosphate-P determination system

2.5.4 Determination of Potassium

The potassium was determined by using a Corning EEL flame photometer.

2.5.4.1 Reagents.

Potassium standard stock solution (1000 mg l^{-1}).

Potassium dihydrogen phosphate was dried at 110°C in the oven. 3.4806 g was dissolved in deionized water and the volume was made to 1 litre.

Potassium working standard solutions were prepared by dilution of stock solution with appropriate extracting solutions.

2.5.4.2 Procedure

The flame photometer was calibrated using 0-25 mg K l^{-1} potassium working standard solutions. The calibration graph in this range is curved. After achieving stable readings, samples were run and their readings were recorded. The calculations were made by a calibration curve programme on a BBC microcomputer in the department.

2.6 EXTRACTION OF INORGANIC NITROGEN FROM SOIL

Ammonium-N, nitrite-N and nitrate-N can be determined from soil solutions after these ions have been extracted from soils by salt solutions. Flowers and Arnold (1983) used 0.5M potassium sulphate during inorganic nitrogen analysis of soil and did not mention any significant interference of the sulphate ion. Potassium sulphate was, therefore, selected for extraction of inorganic nitrogen.

2.6.1 0.5M Potassium Sulphate Preparation

87.125 g potassium sulphate was dissolved in about 800 ml of deionized water and made up to 1 litre. The solution was purified of ammonium-nitrogen contamination by raising its pH to 11.0 with 1M potassium hydroxide. It was then boiled and stirred for 15 minutes to give off ammonia gas. The solution was allowed to cool and the pH was readjusted to pH 6.0 with 0.5M H_2SO_4. Deionized water was added for any loss of water during boiling due to evaporation (Khan, 1987).

2.6.2 Extraction Procedure

An amount of fresh sample from each soil equivalent to 2.5 g on an oven dry basis was weighed into a 4 oz glass bottle and was shaken for 2 hours with 50 ml of 0.5M potassium sulphate. Extraction was done in five replicates in each soil alongwith appropriate blanks. Shaken samples were filtered through washed Whatman filter papers No. 1. The first two to three ml of the filtrates including

blanks were not collected. Filtrates were stored at 2°C in a cold room until analysis (Khan,1987). Ammonium, nitrite and nitrate were determined from these filtrates using the Technicon AutoAnalyzer II.

All the working standard solutions used had equal concentration of 0.5M potassium sulphate as in the soil extracts.

2.7 EXTRACTION OF PHOSPHATE AND POTASSIUM FROM SOIL

There are many extractants such as sodium bicarbonate, sodium acetate, ammonium acetate, ammonium nitrate and acetic acid which are used for the extraction of available phosphate-P and potassium from soils. 0.5M acetic acid solution as an extractant has some merits over other extractants. For example, it gives clear extracts for colorimetric analysis, the same extract can be analysed for phosphate and potassium. Moreover, there is less chance of cross contamination if ammonium extraction is to be carried out in the laboratory at the same time than when ammonium salts are used for phosphorus and potassium extraction. Therefore, it is a favourite extractant used in this department.

2.7.1 0.5M Acetic Acid Preparation

29 ml of glacial acetic acid (AR) was added with a graduated cylinder to approximately 800 ml of deionized water in a 1 litre volumetric flask. Deionized water was added to make volume to the mark.

2.7.2 Extraction Procedure

2.5 g (oven dry basis) of fresh sample of soil was weighed into a 4 oz glass bottle in 5 replicates. 50 ml of 0.5M acetic acid solution was added to each bottle and were shaken for 2 hours on an end-over-end shaker at room temperature. Shaken samples were then filtered through Whatman filter papers No 1. The filtrates were collected including blanks and stored at 2°C until required.

2.8 ACID DIGESTION OF HERBAGE SAMPLES

The grass and root samples were digested by the method of Bremner and Mulvaney (1982). This method includes oxidised forms of nitrogen such as nitrate. However, this method was slightly modified using sodium sulphate-catalyst mixture instead of potassium sulphate-catalyst mixture. This modification facilitated the measurement of nitrogen, phosphorous and potassium in the same digest.

2.8.1 Reagents

Analar grade reagents and nitrogen free deionized water were used throughout.

Salicylic acid-sulphuric acid mixture

25 g salicylic acid was dissolved in 1 litre of concentrated sulphuric acid in a beaker. The mixture was poured carefully into a 1 litre glass bottle fitted with a 5 ml acid dispenser.

Sodium thiosulphate pentahydrate ($Na_2S_2O_3.5H_2O$)

Sodium sulphate-copper sulphate mixture

100 g sodium sulphate was finely ground with 10.0 g of copper sulphate in a mortar and pestle. This mixture was stored in a glass bottle.

2.8.2 Digestion Procedure

Digestion was carried out using a Tecator 40 tube block digester. Digestion tubes were washed with hot water, rinsed several times with deionized water and dried in the oven at 110°C. Approximately 0.2 g finely ground grass and root samples were weighed into digestion tubes. This was done by using a foil weighing boat and the weighing by subtraction method. 5 ml of salicylic acid-sulphuric acid mixture was added to the samples into the digestion tubes. The tubes were swirled to mix the acid with samples. This mixture was left overnight in the fume cupboard without heating. 0.5 g sodium thiosulphate pentahydrate was added into tubes. Then tube holder was placed on the heating block. Gentle heating at 250°C was maintained until frothing ceased. The tubes were removed from the heating block and allowed to cool for 15 minutes. 1.0 g sodium sulphate-copper sulphate mixture was added through a dry plastic funnel with a long stem that reached down into the tube. Tubes were again placed on the heating block and heating at 375°C continued for two hours after the solutions cleared. Tubes were removed from the heating block and it was switched off. After 10-15 minutes cooling, 20 ml deionized water was added to each digest carefully. After cooling, the digests were diluted with deionized water. The final volume was made with deionized water to the mark of 100 ml on the tubes. The digests were filtered through already washed with 0.5M sulphuric acid filter papers Whatman No. 1. The filtered solutions were stored at 2°C in the cold room until required.

2.8.3 Determination of Macronutrients in Digests

Total ammonium-N and phosphate-P contents of the acid digests were determined by colorimetric method using the Tecnicon AutoAnalyzer II. Potassium was determined by Corning EEL flame photometer.

2.8.3.1 Determination of total nitrogen

Acid digests were neutralized with sodium hydroxide prior to reaching the standard ammonium manifold by including an inbuilt neutralization/dilution step.

Reagents

The reagents described in the section 2.5.1 were used in the analysis of acid digests of herbage samples for total ammonium content. The following solutions were also required.

Wash chamber solution (5% v/v)

50 ml concentrated sulphuric acid was diluted to a 1 litre withe deionized water.

Neutralization solution

3.6 g sodium hydroxide (AR) was dissolved in a litre of deionized water.

Ammonium-N working standards (0-100 mg l^{-1})

0 to 100 mg NH_4-N l^{-1} working standard solutions were prepared by diluting the stock ammonium solution with degassed deionized water. All working standard solutions

had same concentration of acid and reagents as in the acid digests. These had 5.0 ml sulphuric acid and 1.0 g sodium sulphate-copper sulphate mixture per 100 ml of solution.

Procedure

The digests were analysed for ammonium-nitrogen using the standard ammonium manifold as shown in Figure 2.1. The dilution ratio was 20:1 of diluent:sample.

Sample	0.1 ml per minute
Diluent	2.0 ml neutralizing solution per minute
Air	0.80 ml per minute

The samples were run at the rate of 40 per hour. The composition of the diluent (neutralizing solution) was checked by sampling some of the wash chamber solution containing a few drops of methyl red indicator. Sodium hydroxide (1M) was added to the neutralizing solution until the indicator changed colour from red to yellow in the first few turns of the diluter mixing coil.

2.8.3.2 Determination of total phosphorus

Acid digests were neutralized with sodium hydroxide prior to reaching the standard phosphate manifold by including an inbuilt neutralization/dilution step.

Reagents

The reagents described in the section 2.5.3 were used in the analysis of acid digests of herbage samples for total phosphate content. The wash chamber solution and

neutralization solution were same as required in the determination of total ammonium (2.8.3.1).

Phosphate working standards (0-50 mg l^{-1})

0 to 50 mg l^{-1} phosphate working standard solutions were prepared by diluting the stock phosphate solution with degassed deionized water. All working standard solutions had same concentration of acid and reagents as in the acid digests. These had 5.0 ml sulphuric acid and 1.0 g of sodium sulphate-copper sulphate mixture.

Procedure

The digests were analysed for phosphate using the standard phosphate manifold as shown in Figure 2.3 with the addition of a neutralization/dilution step. The flow rate of sample, diluent and air were the same as in the total ammonium determination. The samples were run at the rate of 40 per hour. The composition of the diluent was also checked in the same way as in the total ammonium determination.

2.8.3.3 Determination of total potassium

Procedure of potassium determination was followed as described in the section 2.5.4 except that the standard calibration curve covered the range 0-100 mg K l^{-1}. All the working standard solutions had equal concentration of reagents as in the acid digest of samples. These had 5.0 ml sulphuric acid and 1.0 g of sodium sulphate-copper sulphate mixture per 100 ml of solution.

CHAPTER THREE

INTERFERENCE OF ORGANIC NITROGEN COMPOUNDS IN AMMONIUM-N DETERMINATION USING THE BERTHELOT COLOUR REACTION

PART 1 INVESTIGATION OF INTERFERING ORGANIC SUBSTANCES

3.1 INTRODUCTION

Many organic nitrogen compounds are thought likely to occur in soil solutions and extracts. Particularly amino acids are suspected to cause interference in ammonium determination by colorimetric methods. Sowden (1956) found that the amounts of amino acids in soil were in the order glycine > aspartic acid, glutamic acid, alanine > threonine, serine, proline, valine, leucine > isoleucine > tyrosine, phenylalanine > methionine or cystine. Lowe (1973) examined the amino acid distribution in hydrolysates from L, F and H horizons of forest humus layers in British Columbia and reported that amino acid-N content of horizon samples ranged from 0.07% to 0.95%.

The common solutions used for extraction of available nitrogen from soils are either 2M potassium chloride or 0.5M potassium sulphate. The available nitrogen is measured from soil extracts and total nitrogen from Kjeldahl digests by manual or automated techniques (Bremner and Hauck, 1982; Bremner and Mulvaney, 1982 and Keeny and Nelson, 1982). The colorimetric methods of ammonium-nitrogen determination in aqueous solution based on the Berthelot reaction

either catalysed or without catalyst have been reviewed by Searle (1984).

There is evidence about interferences due to the presence of amino acids in samples during nitrogen determination by colorimetric methods from the clinical research. Forgan-Smith et al. (1976) described the Berthelot indophenol reaction as a reliable method for the estimation of ammonia generated by renal tissue slices in Krebs-Ringer bicarbonate sclution containing glutamine provided that the medium is diluted to give a glutamine concentration not exceeding 0.5 mmol/l in the final solution to be assayed.

White and Gosz (1981) reported that amino acids added to the KCl extracts of soil were found to contribute a significant positive interference to the automated determination of ammonium. They modified the Technicon Industrial Method No. 98-70W by using 110 g l^{-1} NaOH instead of 200 g NaOH l^{-1} in the phenol reagent (which lowered the pH to 12.5 in the reagent and 12.0 in the final solution) and by lowering the temperature from 90°C to 60°C in the heating bath for the colour development. Modification of the method yielded results identical to steam distillation analysis. The original method was found to overestimate inorganic ammonium-nitrogen in extracts of forest floor material by 17-26%. Rowland (1983) concluded from his study that amino acids interfere, but only contribute a small error (11%) in soil extractable nitrogen.

Reaction conditions can change the extent of interference by amino acids and the sensitivity of the specific method of ammonium-nitrogen using the Berthelot reaction. For instance, very low hypochlorite concentrations may result in negative interference through reactions between amino acids and the hypochlorite source (Searle, 1984). Adamsen et al. (1985) determined ammonium, nitrite and nitrate from 2M KCl-Phenylmercuric acetate extracts of soil. They used salicylate-nitroprusside as the phenolic compound, dichloroisocyanurate as the chlorine donating agent and NaOH. They reported that glycine and creatine gave apparent ammonium recovery, this would be due to increased breakdown of these compounds in the higher pH of the proposed reagents. Burton et al. (1989) found small but significant errors due to amino acid interference in the ammonium determination by automated method for soil extracts.

Searle (1990) concluded that the Berthelot reaction can be used for measuring ammonium ions in the presence of appreciable concentrations of amino acids, provided that the reagents and reaction conditions used are carefully chosen to limit hydrolysis. This is best achieved by using the nitroprusside catalysed reaction, which enables the use of low sample volume, low reagent concentrations and low reaction temperatures.

Keeping in view the above facts, it was, therefore, decided to conduct experiments to investigate the possible interference by organic nitrogen compounds in

combination with known concentration of ammonium-nitrogen in water, 2M potassium chloride and 0.5M potassium sulphate.

3.2 METHODS AND MATERIALS

3.2.1 Washing of Glassware

As in section 2.1.

3.2.2 Purification of 2M Potassium chloride and 0.5M Potassium sulphate

As in section 2.6.1

3.2.3 Determination of Ammonium-N

As in section 2.5.1.

3.2.3.1 Reagents

As in section 2.5.1.1 and the remaining reagents were as follows:-

Organic compounds

The organic compounds studied for their possible interferences in ammonium-N determination in water, 2M potassium chloride and 0.5M potassium sulphate are shown in Table 3.1.

Table 3.1 Number of nitrogen atoms and molecular weight of organic nitrogen compounds.

Organic compounds	Number of nitrogen atoms	Molecular Weight
Alanine	1	89.09
Arginine	4	210.70
Asparagine	2	150.10
Aspartic acid	1	133.10
Glutamic acid	1	147.10
Glutamine	2	146.10
Glycine	1	75.07
Histidine	3	209.60
Isoleucine	1	131.20
Leucine	1	131.20
Lysine	2	183.70
Methionine	1	149.20
Phenylalanine	1	165.20
Proline	1	115.10
Serine	1	105.10
Threonine	1	119.20
Valine	1	117.10
Galactosamine	1	215.60
Glucosamine	1	215.60
Urea	2	60.06

Working solutions of organic nitrogen compounds

The required weights of 17 amino acids, galactosamine, glucosamine and urea were transferred to 25 ml volumetric flasks carefully and volume was made to the mark with deionized water. The strength of each solution was 1000 mg N l^{-1}

NH_4-N stock solution (1000 mg N l^{-1})

As in section 2.5.1.1

Ammonium-N working solution (100 mg N l^{-1})

10 ml of 1000 mg l^{-1} NH_4-N stock solution was pipetted into 100 ml flask by a bulb pipette and the volume was made with deionized water up to the mark.

Ammonium-N working standards (1 mg N l^{-1})

1 ml of 100 mg l^{-1} NH_4-N working solution was added to each of three 100 ml volumetric flasks and the volume was made with deionized water, 2M KCl and 0.5M K_2SO_4, respectively.

Zero ammonium-N working solutions

Deionized water, 2M KCl and 0.5M K_2SO_4 were analysed as zero ammonium-N solutions with their appropriate set of samples.

Organic compound samples without added ammonium-N

1 ml of each organic nitrogen compound stock solution (1000 mg N l^{-1}) was diluted separately into 100

ml volumetric flasks with water, 2M KCl and 0.5M K_2SO_4. Each solution contained 10 mg N l^{-1} .

Organic compound sample with added ammonium-N

1 ml of ammonium-N (100 mg N l^{-1}) and 1 ml of organic compound solution (1000 mg N l^{-1}) were added together by an automatic pipette into three 100 ml volumetric flasks for each compound. These were then diluted with water, 2M KCl and 0.5M K_2SO_4 to produce solutions containing 1 mg l^{-1} NH_4-N and 10 mg l^{-1} organic nitrogen.

3.2.3.2 Procedure

The ammonium-N manifold shown in figure 2.1 was used for ammonium-N determination in water, 2M KCl and 0.5M K_2SO_4 and also to determine the possible interference of organic nitrogen compounds. Procedure in section 2.5.1.2 was followed in the determination of NH_4-N in organic N solution with and without NH_4-N in water, 2M KCl and 0.5M K_2SO_4 using their appropriate 0 and 1 mg l^{-1} NH_4-N working standards and blanks solutions.

3.3 RESULTS AND DISCUSSION

3.3.1 Preliminary Investigations of Interferences caused by Amino acids in Ammonium-N Determination

Interferences by amino acids

Preliminary experiments were carried out to investigate if there is an effect of any of the selected amino acids on ammonium-N determination. These results are shown in Table 3.2.

Table 3.2 Interferences by amino acids in ammonium-N determination.

	Effect in (mg l^{-1}) N of organic compounds at an ammonium concentration of					
	0	1 (mg l^{-1})	0	1 (mg l^{-1})	0	1 (mg l^{-1})
Amino acids	Water		2M KCl		0.5M K_2SO_4	
Alanine	0.12*	-0.04**	0.14	-0.08	0.12	-0.17
Glutamic acid	0.02	-0.02	0.09	-0.10	0.07	-0.17
Glycine	0.21	-0.03	0.24	-0.10	0.12	-0.22
Histidine	0.04	-0.03	0.02	-0.04	0.02	-0.07
Methionine	0.10	-0.16	0.02	-0.26	0.01	-0.35
Phenylalanine	0.05	-0.06	0.05	-0.11	0.03	-0.15
Proline	0.09	-0.02	0.03	-0.08	0.02	-0.15
Serine	0.10	-0.05	0.09	-0.11	0.06	-0.18
Threonine	0.10	-0.05	0.08	-0.10	0.06	-0.18
Valine	0.03	-0.02	0.03	-0.10	0.02	-0.19

* Each value is the mean of two replicates.

** This is a corrected value by deducting the interference (mean of two replicates) by each amino acid at 0 mg l^{-1} N.

It is clear from Table 3.2 that all amino acids showed positive interference at zero ammonium-N concentration. Glycine showed greater interference than all others amino acids in water and 2M KCl. It remained equal with alanine in K_2SO_4. It indicates that reaction conditions are influencing breakdown of these amino acids.

Amino acids in water and soil extracting solutions (2M KCl and 0.5M K_2SO_4) showed negative interferences at 1.0 mg l^{-1} NH_4-N. Methionine suppressed most strongly the colour development due to added NH_4-N. This resulted in the negative interference of -0.16, -0.26 and -0.35 in water, 2M KCl and 0.5M K_2SO_4, respectively. It is very clear that the negative interferences by amino acids has increased in the salt solutions commonly used in soil extracting research work. Probably this is due to the effect of chloride and sulphate ions on the chemistry of the Berthelot reaction. This confirms the suspicion that amino acids present in samples analysed by colorimetric methods could result in over or underestimate of NH_4-N in soils.

Variation in interferences between two analysis

The same test solutions were analysed twice, a few days apart using freshly prepared and slightly aged reagents. The results of the two set of analysis using five amino acids in three extracting solutions are shown in Table 3.3.

The results show variation in the interferences by amino acids between analysis dates at zero and 1.0 mg l^{-1}

added ammonium-N. Glycine showed greater positive interference at zero ammonium concentration.

Table 3.3 Variation in interferences by amino acids at two analysis dates.

	Effect in (mg l^{-1}) N of organic compounds at an ammonium concentration of			
	0 (mg l^{-1})	0	1 (mg l^{-1})	1
	run 1	run 2	run 1	run 1
Amino acids in Water				
Alanine	0.20*	0.09	-0.03**	+0.01
Glycine	0.20	0.12	-0.08	-0.01
Phenylalanine	0.06	0.05	+0.07	-0.03
Proline	0.05	0.03	+0.04	+0.01
Threonine	0.13	0.11	-0.07	-0.03
Amino acids in 2M potassium chloride				
Alanine	0.07	0.07	-0.04	-0.06
Glycine	0.10	0.08	-0.06	-0.08
Phenylalanine	0.02	0.04	-0.05	-0.10
Proline	0.01	0.01	-0.04	-0.07
Threonine	0.08	0.08	-0.07	-0.10
Amino acids in 0.5M potassium sulphate				
Alanine	0.06	0.06	-0.12	-0.09
Glycine	0.07	0.08	-0.10	-0.15
Phenylalanine	0.03	0.04	-0.14	-0.19
Proline	0.01	0.06	-0.13	-0.09
Threonine	0.06	0.08	-0.08	-0.23

* Each value is the mean of two replicates.

** This is a corrected value after deducting the interference (mean of two replicates) by each amino acid at 0 mg l^{-1} N.

These results lead to the point of important consideration that reaction conditions have an effect on interferences caused by amino acids. The inconsistency in the results of same test solutions analysed on separate dates suggest that before conducting the main experiment, it will be necessary to investigate the effect of reaction conditions on the level of interferences by amino acids in ammonium-N determination.

3.3.2 Investigation of Reaction Conditions

Keeping in view the inconsistency in the results of two analysis of same test solutions, it was necessary to optimize those conditions, which may have an effect on the Berthelot indophenol colour reaction.

Effect of reagent age

Solutions of three amino acids (chosen for their large interferences in water, see Table 3.2) were studied for their interferences in ammonium-N determination using one day and seven days old reagents. The results obtained are presented in Table 3.4. Evidently the age of the reagents did not have much effect on the interferences by amino acids in ammonium determination. However, the freshly prepared reagents gave more reproducible results.

Table 3.4 Effect of reagent age on interferences caused by amino acids.

		Effect in (mg l^{-1}) N of organic compounds at an ammonium concentration of					
		0 (mg l^{-1})	1	0 (mg l^{-1})	1	0 (mg l^{-1})	1
Reagent age (days)	Amino acids	Water		2M KCl		0.5M K_2SO_4	
1	Serine	0.03*	-0.01**	0.02	-0.04	0.02	-0.05
	Glycine	0.07	-0.01	0.05	-0.08	0.05	-0.10
	Proline	0.02	-0.01	0.00	-0.13	0.00	-0.07
7	Serine	0.04	-0.01	0.03	-0.02	0.03	-0.03
	Glycine	0.10	-0.01	0.07	-0.06	0.07	-0.04
	Proline	0.04	0.00	0.02	-0.12	0.02	-0.03

* Each value is the mean of two replicates.

** This is a corrected value by deducting the interference (mean of two replicates) by each amino acid at 0 mg l^{-1} N.

Effect of temperature

The effect of temperature 0, 20, 40 and 60°C was evaluated for its influence on the interferences due to amino acids in ammonium-N determination. The temperature was arranged by water from a controlled temperature water bath flowing through a 1.2 cm plastic pipe. The reagents lines were wrapped around this pipe several times to ensure that the reagents reached the required temperature before entering the system manifold. The temperature of 20, 40 and 60°C were obtained through the water bath thermostat. 0°C was maintained by adding ice to the water bath. The results are shown in Table 3.5.

Table 3.5 Effect of reagent temperature on interferences caused by amino acids.

		Effect in (mg l^{-1}) N of organic compounds at an ammonium concentration of					
		0 (mg l^{-1})	1	0 (mg l^{-1})	1	0 (mg l^{-1})	1
Temp. (°C)	Amino acids	Water		2M KCl		0.5M K_2SO_4	
0	Serine	0.03*	-0.04**	0.03	-0.02	0.03	-0.02
	Glycine	0.10	-0.02	0.10	-0.04	0.10	-0.02
	Proline	0.02	+0.01	0.02	-0.10	0.02	-0.01
20	Serine	0.04	+0.01	0.04	-0.04	0.03	-0.03
	Glycine	0.10	-0.03	0.07	-0.08	0.07	-0.07
	Proline	0.03	-0.01	0.02	-0.13	0.01	-0.05
40	Serine	0.04	-0.01	0.02	-0.10	0.02	-0.09
	Glycine	0.08	-0.03	0.04	-0.19	0.04	-0.16
	Proline	0.02	-0.02	0.00	-0.23	0.00	-0.15
60	Serine	0.05	-0.06	0.01	-0.24	0.00	-0.18
	Glycine	0.08	-0.08	0.00	-0.63	0.00	-0.38
	Proline	0.02	-0.05	0.00	-0.44	0.00	-0.40

* Each value is the mean of two replicates.

** This is a corrected value by deducting the interference (mean of two replicates) caused by each amino acid at 0 mg l^{-1} N.

At zero ammonium-N, the amino acid positive interference remained almost unaffected in water, but it was reduced in salt solutions with increase in temperature. However, an increase in temperature resulted in increased negative interferences by amino acids at 1.0 mg l^{-1} ammonium-N in potassium chloride and sulphate

solutions but not in water. These results point out that any fluctuation in room temperature will lead to the variation in the results. This problem of inconsistency in the results can be overcome by placing the reagent bottles in a water bath with a constant temperature of 25°C.

Effect of hypochlorite concentration

The effect of two hypochlorite concentrations is shown in Table 3.6.

Table 3.6 Effect of hypochlorite concentrations on interferences caused by amino acids.

		Effect in (mg l^{-1}) N of organic compounds at an ammonium concentration of					
		0 (mg l^{-1})	1	0 (mg l^{-1})	1	0 (mg l^{-1})	1
Amino acids	Hypochlorite (ml l^{-1})	Water		2M KCl		0.5M K_2SO_4	
Alanine	25	0.02*	-0.03**	-0.05	-0.34	-0.00	-0.31
Glycine		0.05	-0.05	-0.03	-0.35	0.02	-0.33
Threonine		0.01	-0.04	-0.06	-0.31	-0.02	-0.31
Phenylalanine		0.00	-0.05	-0.07	-0.33	-0.03	-0.32
Proline		0.00	-0.04	-0.06	-0.33	-0.02	-0.29
Alanine	50	0.09	-0.02	0.09	-0.04	0.08	-0.05
Glycine		0.12	-0.03	0.09	-0.05	0.09	-0.08
Threonine		0.12	-0.03	0.05	-0.04	0.09	-0.09
Phenylalanine		0.05	-0.04	0.05	-0.07	0.04	-0.09
Proline		0.03	-0.01	0.02	-0.03	0.02	-0.05

* Each value is the mean of two replicates.

** This is a corrected value by deducting the interference (mean of two replicates) caused by each amino acid at 0 mg l^{-1} N.

Two solutions of hypochlorite reagent were prepared by dissolving separately 25 ml and 50 ml hypochlorite in a litre of deionized water. The same test solutions of amino acids were run sequentially using these hypochlorite

solutions. All other conditions and reagents were kept constant. The results of the two hypochlorite concentrations shown in Table 3.6 indicate that amino acids at zero ammonium-N addition caused small negative interferences in salt solutions at low hypochlorite concentration. In water, however, their interferences were positive. Only glycine showed a positive interference in potassium sulphate. Use of 50 ml per litre hypochlorite resulted in positive interferences by these amino acids at zero ammonium-N concentration in all three solutions.

At 1.0 mg l^{-1} ammonium-N, all the amino acids interfered negatively at both hypochlorite concentrations. Addition of amino acids to all three solutions at 1.0 mg l^{-1} ammonium-N and low hypochlorite concentration resulted in strong negative interferences. At 50 ml hypochlorite per litre, these negative interferences were much reduced. It can be suggested from these results that 50 ml per litre of hypochlorite is the optimum rate to be used. Possibly, some of variations in the early results could be due to the deterioration of the hypochlorite reagent.

3.3.3 Summary about Interferences by Amino acids and Reaction Conditions

The following points can be concluded from these experiments:-

Amino acids cause interferences in ammonium-N determination by the Berthelot indophenol colour reaction.

Freshly prepared reagents should be used in studies investigating interferences by amino acids.

Room temperature should be stable or reagents bottles should be placed in a water bath having a constant temperature of 25°C.

The hypochlorite reagent should be freshly prepared at a concentration of 50 ml per litre.

3.3.4 Interferences by Organic Nitrogen Compounds in Ammonium-N Determination

After investigating the reaction conditions, the main experiment was conducted to investigate the interferences by 17 amino acids, galactosamine, glucosamine and urea. These compounds were evaluated for interferences in ammonium-N determination in water, potassium chloride and potassium sulphate solutions at ammonium-N level of 0 and 1.0 mg l^{-1}. The results are presented in Table 3.7.

Table 3.7 Interferences by organic nitrogen compounds.

	Effect in (mg l^{-1}) N of organic compounds at an ammonium concentration of					
	0 (mg l^{-1})	1	0 (mg l^{-1})	1	0 (mg l^{-1})	1
Organic compounds	Water		2M KCl		0.5M K_2SO_4	
Glycine	0.03*	-0.09**	0.03	-0.21	0.03	-0.21
Alanine	0.02	-0.07	0.02	-0.19	0.02	-0.19
Valine	0.00	-0.03	0.00	-0.10	0.00	-0.13
Leucine	0.01	0.00	0.00	-0.09	0.00	-0.07
Isoleucine	0.00	-0.03	0.00	-0.10	0.00	-0.12
Serine	0.02	-0.05	0.01	-0.12	0.01	-0.14
Threonine	0.00	-0.08	0.00	-0.19	0.00	-0.20
Methionine	0.00	-0.23	0.00	-0.38	0.00	-0.40
Aspartic acid	0.04	0.00	0.04	-0.09	0.08	-0.08
Glutamic acid	0.03	-0.05	0.03	-0.18	0.02	-0.14
Asparagine	0.01	-0.01	0.02	-0.04	0.01	-0.04
Glutamine	0.02	0.00	0.02	-0.03	0.02	-0.03
Lysine	0.02	-0.04	0.02	-0.09	0.02	-0.09
Arginine	0.00	-0.01	0.00	-0.04	0.00	-0.02
Phenylalanine	0.00	-0.12	0.00	-0.13	0.00	-0.16
Proline	0.00	-0.07	0.00	-0.18	0.00	-0.19
Histidine	0.00	-0.03	0.00	-0.04	0.00	-0.06
Urea	0.02	+0.02	0.09	+0.01	0.10	+0.02
Galactosamine	0.17	-0.01	0.09	-0.07	0.10	-0.06
Glucosamine	0.02	-0.02	0.01	-0.06	0.01	-0.06

* Each value is the mean of two replicates.

** This is a corrected value by deducting the interference (mean of two replicates) caused by each amino acid at 0 mg l^{-1} N.

The results in Table 3.7 show positive interferences by amino acids at 0 mg l^{-1} ammonium-N addition in water, potassium chloride and potassium sulphate. These interferences ranged from 0 to 0.17 mg l^{-1}. These results show positive interference by some amino acids while

others did not show colour development using the Berthelot reaction. The positive interferences might be due to either ammonium-N impurities in the amino acid samples, their participation in the reaction or their hydrolysis under the reaction conditions to produce ammonium-N. However, it is not possible to distinguish between positive chemical interferences and ammonium-N impurities. Amino acids with small molecular weights showed small positive interferences which disappeared in the amino acids with the larger molecular weights (either branched or straight chain). The amino acid sample solutions were prepared on the basis of molecular weight divided by the number of N atoms in their molecules. Therefore, all these solutions had same molarity. However, it is not as simple to make a clear comparison of the concentrations of amino acids such as glycine and arginine which contain different forms of N in their molecules.

There are some effects of the functional groups. For instance, the comparison between the carboxylic acid and amide containing amino acids shows that the carboxylic acids caused more interference than the corresponding amides. The ring structures either aromatic or heterocyclic showed no interference at all. The largest interference which was due to galactosamine again might be due to either its hydrolysis or ammonium-N impurities.

These results lead to the conclusion that there is no breakdown of the amino acids under the present reaction conditions as the interferences are shown by the short chain unsubstituted amino acids such as glycine and not by

those having functional groups which might more easily hydrolyse to yield ammonium-N such as lysine or glutamine.

The addition of the amino acids, galactosamine and glucosamine caused negative interferences at 1.0 mg l^{-1} ammonium-N in water, potassium chloride and potassium sulphate. Only urea interfered positively. There was little difference in its interference between water and the salt solutions. However, in the case of the amino acids, negative interference increased between water and the salt solutions. The negative interferences by the unsubstituted amino acids were inversely proportion to their molecular weights (glycine to isoleucine, see Table 3.7). On the other hand, the interferences by the amino acids having hydroxyl or carboxylic groups in their molecules such as serine, threonine, aspartic and glutamic acids increased with an increase in the molecular weight. As far as the effect of amides on the interference is concerned, the amino acids with amide groups showed less interference than the corresponding carboxylic acids. Of the five remaining amino acids, arginine and histidine showed low negative interferences compared with lysine, proline and phenylalanine. Galactosamine and glucosamine showed almost similar interferences at this concentration of ammonium-N. Methionine was the amino acid which suppressed the colour development most strongly in this study. It inhibited colour formation by up to 23% in water, 38% in 2M potassium chloride and 40% in the 0.5M potassium sulphate.

These results show that many of the amino acids, and particularly methionine, if present in soils and extracted by potassium salts can cause errors of up to 40% in the determination of available ammonium-N by the Berthelot reaction. There is evidence of larger interferences by the amino acids in the literature. White and Gosz (1981) compared an automated indophenol method (Technicon Industrial Method No. 98-70W) with steam distillation (Bremner, 1965) for the determination of ammonium-N in 2M KCl extracts of forest floor samples. They found that amino acids in 2M KCl contributed significant positive interferences to the automated method. This method overestimated ammonium-N in extracts from 17-26%. To minimize the interferences by the amino acids, they modified the method by using 110 g l^{-1} sodium hydroxide instead of 200 g l^{-1} in the phenol reagent (which lowered the pH to 12.5 in the reagent and to 12.0 in the final solution). They also lowered the temperature from 90°C to 60°C for the colour development step. Their modification yielded results identical to steam distillation analysis.

Rowland (1983) reported that amino acids such as glycine, glutamic acid, alanine, leucine and aspartic acid at a concentration of 1 mg l^{-1} interfered in the ammonium-N determination by the nitroprusside catalysed indophenol reaction at low temperature (Technicon Industrial Method No. 329-74W) and showed an apparent recovery compared with steam distillation of up to 47% of the amino acids nitrogen. He concluded from these results that interference was not due to contamination of ammonia in

the reagent but the breakdown of the amino acids in solution.

He also found levels of interference which ranged from 1.3 to 17.1% in the extracts of 19 soils having low ammonium content (below 4.0 mg l^{-1} extractable ammonium-N). There were significant correlations between the interference level and the loss on ignition and the total nitrogen content of the soil. He concluded that release of ammonia due to breakdown of organic fractions in soil extracts seem to occur at the temperature and pH of nitroprusside catalysed reaction. Attempts to reduce the interference levels by adjusting the pH of the reaction, inclusion of a buffer or by reduction of the heating period in the colour formation step did not produce significant reduction in the interference.

Burton et al. (1989) analysed amino acids in 2M KCl solutions containing ammonium-N at 20 mg l^{-1} by both an automated Berthelot procedure (Technicon Industrial Method No. 98-70W) and by distillation with magnesium oxide (Bremner, 1965). They also analysed 2M KCl soil extracts by both steam distillation and an automated indophenol method. Less than 2% of the organic nitrogen present in the various solutions was detected as ammonium by steam distillation. With the automated indophenol procedure, the apparent recovery of amino nitrogen as ammonium varied from 0 to 94%. Except for threonine, the percentage apparent recovery tended to be inversely related to the molecular weights of the amino acids.

The automated indophenol method compared with steam distillation showed apparent recovery as ammonium in 2M KCl extracts of soil samples. Amino acid interference may be particularly important in perturbed soil samples and in studies of the inter-conversion of organic and inorganic forms of nitrogen. Therefore, they recommended distillation for ammonium determination in such samples to avoid over or under estimation of the different forms of nitrogen.

Searle (1990) suggested that the Berthelot reaction can be used for measuring ammonium ions in the presence of appreciable concentration of amino acids, provided that the reagents and reaction conditions used are carefully chosen to limit hydrolysis. He suggested that this is best achieved by using the nitroprusside catalysed reaction, which enables the use of low sample volumes, low reagent concentrations and low reaction temperatures.

The present method (Brown, 1973) is a nitroprusside catalysed indophenol reaction at a low temperature of 37°C. The sodium hydroxide concentration in the phenol reagent was 22.5 g l^{-1} and in the final reaction mixture solution was 8.0 g l^{-1}. Moreover, Table 3.8 shows the comparison of the reaction conditions of the different methods as discussed above.

Table 3.8 Comparison of different reaction conditions.

Source	Method	Temperature (°C)	NaOH in Reagent ($g\ l^{-1}$)	NaOH in Final solution ($g\ l^{-1}$)
White and Gosz (1981)	Technicon Idustrial Method 98-70-W	90	200	41
White and Gosz (1981)	Technicon Idustrial Method 98-70W Modified	60	110	22
Rowland (1983)	Technicon Industrial Method 329-74W	37	20	13
Burton et al. (1989)	Technicon Idustrial Method 98-70W	90	200	41
Searle (1990)	Technicon Idustrial Method 98-70W	90	200	41
Searle (1990)	Method A	45	45	11
Searle (1990)	Method B	30	10	5
Brown (1973)	Present Method	37	22.5	8

The above quoted research work indicated that the amino acids or other organic compounds are more or less hydrolysed due to the high pH of the reagents in the nitroprusside catalysed reaction and the high temperature in the methods without a nitroprusside catalyst. The results show that hydrolysis during the Berthelot colour reaction results in the release of ammonia which causes

errors in the determination of ammonium-N of soil extracts and other biological samples. The modifications of the methods did not eliminate the positive interferences. However, only the methods of Searle (1990) reduced the positive interferences to much extent. No one has tried to investigate the inhibition effect of amino nitrogen on the ammonium recovery when added to certain ammonium-N concentration except White and Gosz (1981). The results of the present study suggest that the hydrolysis rate is very low compared with the lowest hydrolysis of amino acids reported by Searle (1990), see Table 3.9. Therefore, it can be assumed that amino acids are not hydrolysed during the Berthelot colour reactions unless the amino group on the α-carbon is more readily hydrolysed in the low molecular weights amino acids. Table 3.9 further explains that organic compounds are not hydrolysed during the present method (Brown, 1973).

Table 3.9 Percentage recovery of amino acid nitrogen as ammonium-N.

Nitrogen source amino acids	Technicon* method 98-70W	Method[1] Searle A	Method[2] Searle B	Method[3] Brown
Threonine	94	22	4	0.0
Glycine	70	25	4	0.3
Alanine	36	15	1	0.2
Glutamic acid	19	11	1	0.3
Methionine	17	11	1	0.0
Leucine	12	6	1	0.0
Phenylalanine	10	12	1	0.0

*. Burton et al. (1989). The value is % apparent recovery of 20 mg N l^{-1}.

1. Searle (1984). The value is % apparent recovery of 20 mg N l^{-1}.

2. Searle (1990). The value is % apparent recovery of 20 mg N l^{-1}.

3. Brown (1973). Method followed in present study. The value is % apparent recovery of 10 mg N l^{-1}.

The results presented in Table 3.7 show that addition of the amino acids to 1.0 mg l^{-1} ammonium-N caused a negative interferences in water, 2M KCl and 0.5M K_2SO_4. This effect of the amino acids on the apparent recovery of ammonium-N seems worse in the potassium salt solutions compared with water. The amino acids present in the ammonium-N solution inhibited the Berthelot colour formation acting as reaction inhibitors, for example methionine inhibited the ammonium colour formation up to 40%.

It can be summarised from these results that organic nitrogen compounds interfere negatively in ammonium-N determination using the nitroprusside catalysed Berthelot reaction by the Technicon AutoAnalyzer II. Soil extracting solutions like 2M potassium chloride and 0.5M potassium sulphate further increase interferences by these compounds. These results suggest that a pre-treatment step either distillation or gas phase dialysis should be included to reduce the interferences caused by the amino acids in the ammonium-N determination by the Technicon AutoAnalyzer II.

PART 2 DEVELOPMENT OF A DIALYSIS SYSTEM

3.4 INTRODUCTION

The results summarized in the part 1 show that there are chemical interferences caused by certain organic compounds in the ammonium-N determination by the automated Berthelot reaction. There is a need of a pre-treatment to be included with the Technicon AutoAnalyzer II method for elimination of chemical interferences caused by the organic compounds in the ammonium-N determination.

The ammonium-N can be separated from interferences either by distillation or gas phase dialysis. Crowther and Evans (1980) proposed an automated distillation-spectrophotometry procedure for determining ammonium in water describing that the distillation pre-treatment step for ammonium analysis was automated and coupled with an automated phenate-hypochlorite spectrophotometric step. Two buffers (phosphate, pH 7.4, and borate, pH 9.5) were evaluated as pH controls for the distillation step; with both buffers hydrolysis of 24 organic nitrogen compounds was less than 0.5% and the recoveries of added ammonia from solutions of 55 test compounds were at least 95%. The calibration for the system was linear although the analysis rate was slow (20 samples per hour).

Recent research has been focused on flow injection analysis (FIA) rather than continuous flow analysis (CFA). The gas dialysis using a polytetrafluroethene (PTFE) gas permeable membrane as pre-treatment in the

determination of nitrogen by FIA has become a method of wide use in the research laboratories throughout the world. Van der Linden (1983) has tested several microporous hydrophobic membranes for the determination of ammonia in freshwater systems. Aoki et al. (1986) used tubular microporous PTFE in a continuous flow system. They separated the ammonia generated in alkaline sample solution with this membrane. Martin and Meyerhoff (1986) described a method for using a PTFE membrane in a home made dialysis with wider channels instead of commercially available dialyser in a flow injection system. They were developing a highly selective semi-automated method for the determination of dissolved NO_x or nitrite at levels greater than 5 μM. They noted that nitrogen dioxide is transferred across a teflon membrane in the dialysis and converted to nitrate by a buffered peroxide recipient solution. They concluded that a dialysis with wider channels improves the efficiency of gas transfer and thus the detection capabilities of the system. Willason and Johnson (1986) described a procedure based on the conversion of ammonium-N in sea water to ammonia and the subsequent diffusion of ammonia across a hydrophobic membrane using flow injection analysis. Hara et al. (1988) developed a simple concentrator based on a microporous PTFE membrane and polymer nets and combined it with an ammonia gas electrode to construct a continuous flow determination system for low concentration of ammonium ions in water. They reported that gas dialysis concentrator continuously gave about a

ten fold increase in the concentration of ammonium ions. They applied this system to the determination of residual concentrations of ammonium ions in water purified by distillation or de-ionisation and to natural water analysis. Nakata et al. (1988) studied in detail the spectrophotometric determination of ammonium ion in water by flow-injection analysis with a membrane-separator and a pH indicator for detection. They found that bromocresol purple (pH 6.8) as acceptor solution gives the maximal sensitivity in the flow system with a laboratory-made separation unit. The use of different flow rates for the donor and acceptor streams may result in increased permeation of ammonia and a correspondingly high sensitivity. By modifying the acceptor solution so that the sensitivity is decreased, more concentrated samples such as urine can be analysed by direct injection without prior dilution. Schulze et al. (1988) criticised the routine methods such as Nesslerization and the Berthelot reaction for determining submicromolar levels of ammonium in sea-waters, certain surface waters, arctic and glacial ice. They proposed that gas separation is more satisfactory. Van Staden and Rensburg (1990) evaluated the commercially available semi-permeable membranes for use with parallel-plate dialyses in flow injection system. They reported that the fraction of analyte transferred from the donor to the acceptor stream depends on parameters such as the type of membrane used, membrane surface, membrane line-length, membrane porosity, concentration of analyte in the donor stream, the use of

concurrent and countercurrent flow between the donor and acceptor streams, and flow rates of the donor and acceptor streams.

These characteristics of PTFE gas dialysis may affect the performance of the CFA method, for example if included with the Technicon AutoAnalyzer II system of ammonium determination. Therefore, it seems appropriate to develop a dialysis system which could be included with ammonium-nitrogen system manifold of the Technicon AutoAnalyzer II.

3.5 METHODS AND MATERIALS

3.5.1 Washing of Glassware

As in section 2.1.

3.5.2 Determination of Ammonium-N

As in section 2.5.1.

3.5.3 Reagents

As in section 3.2.3.1 and the remaining reagents were as follows:-

Organic compounds

The organic compounds studied for their possible interferences in ammonium-N determination in water have already been shown in Table 3.1.

1M sodium hydroxide

40.0 g sodium hydroxide was dissolved in approximately 500 ml of deionized water in a 1 liter volumetric flask. After cooling, the volume was made up to the mark with deionized water.

2M hydrochloric acid

170 ml concentrated hydrochloric acid was diluted with deionized water and volume was made up to the mark in a 1 liter volumetric flask.

1M hydrochloric acid

85 ml concentrated hydrochloric acid was diluted with deionized water in a 1 liter volumetric flask up to the mark.

0.01M hydrochloric acid

10 ml of 1M hydrochloric acid were pipetted into a 1 liter volumetric flask and volume was made up to the mark with deionized water.

3.5.4 Materials

Gas dialysis membranes

Tetrafluoroethene (TFE) 1" x 600

This was a product (Part No. 14-831-3006) of Fisher Scientific Co; USA.

Polytetrafluoroethene (PTFE) 1" x 600

This was a product (Military specification) of Gore-Tex W.L. and Associates, INC; Newark, Delaware, USA.

Technicon silicon rubber membrane

This was a product (Product No. 157-13129) of the Technicon Corporation, New York, USA.

Dialysis blocks

These were 3" and 6" dialyzers of the Technicon Corporation, NewYork, USA.

3.6 RESULTS AND DISCUSSION

3.6.1 Ammonium-N Determination by including Dialysis with Technicon AutoAnalyzer II

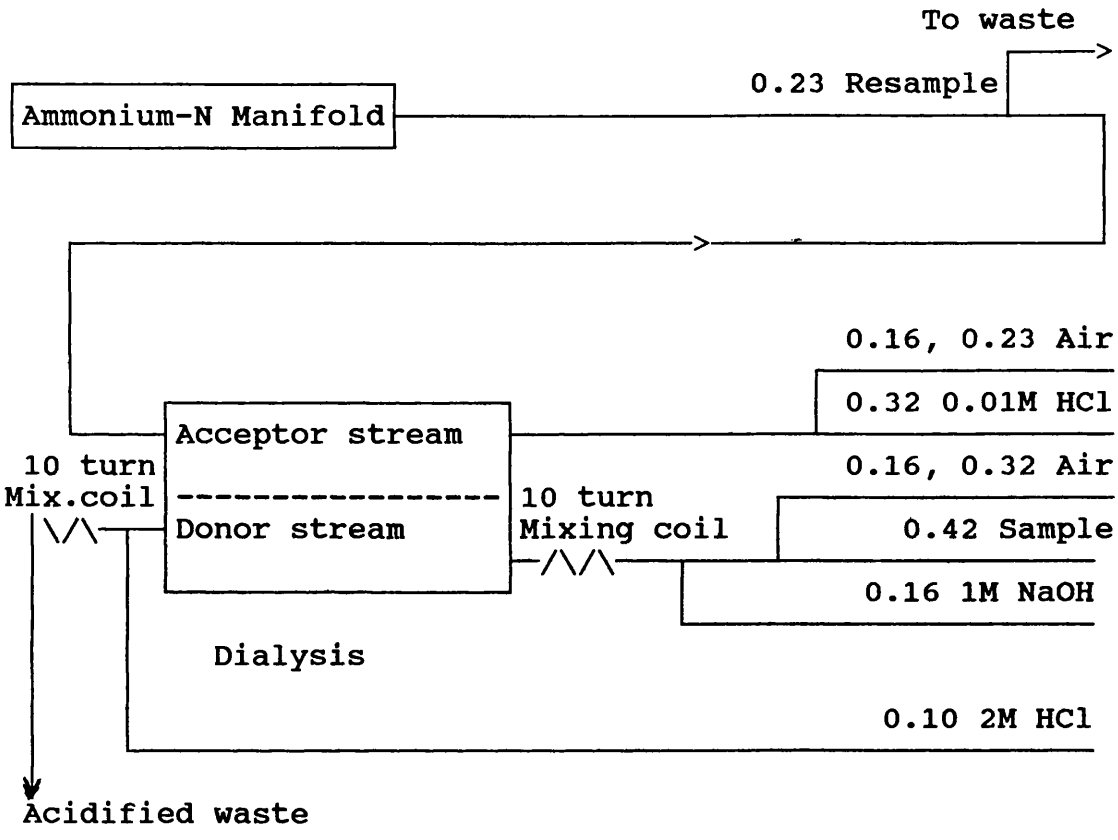

Figure 3.1 Original dialysis system.

The dialysis system shown in the Figure 3.1 worked on the mechanism explained below:-

The donor stream of the dialysis was alkaline, therefore, the ammonium in the sample was converted to NH_3 which passed across the membrane and trapped in the acid acceptor stream and again converted to NH_4-N. As there is liquid and air bubbles in the donor stream, it is likely

to happen that ammonia either can pass directly from the liquid across the membrane or first move into the air bubbles and then pass through the membrane. It is also possible that NH_3 adopts both paths for crossing the membrane.

Initial tests were carried to investigate the effect of concentration of the acceptor solution on the chemistry of ammonium-N main manifold. These tests showed that 0.1M hydrochloric acid affected the chemistry of the main manifold as shown in the Table 3.10. Therefore, 0.01M hydrochloric acid was used in the optimization of the dialysis system for further work.

Table 3.10 The effect of sample acid concentration on the measurement of NH_4-N by the main manifold.

NH_4-N (1 mg l^{-1}) in	NH_4-N measured
Water	1.00
0.01M HCl	1.01
0.1M HCl	0.80

Initial tests carried out by using the system shown in the Figure 3.1 did not show smooth flow. Peaks were not reproducible. Adding wetting agent (Brij-35) in the donor and acceptor streams showed some improvements. Mass flow of air across the dialysis membrane disturbed the air bubble pattern. Therefore, the air bubbles were eliminated from the acceptor side of the dialysis.

3.6.2 Optimization of the Flow rates of Acceptor and Donor Streams of the Dialysis

The changes made in the original dialysis system shown in the Figure 3.1 resulted in the system shown in the Figure 3.2. The main objective of the experiments described in this section was to find out the best flow rates of air, acid, sodium hydroxide and sample through the donor and acceptor streams of the dialysis system.

Air elimination from the acceptor side of the dialysis improved the flow. The flow stabilized and the peak shape improved. However, the ammonium recovery was low. Therefore, the optimization of the flow rates through acceptor and donor sides of the dialysis was carried out using balanced and unbalanced flows.

Some of the possible combinations of the following flow rates of the different parameters were tested using the system 2 shown in the Figure 3.2 and the results are shown in Table 3.11.

Air flow = 0.32, 0.60 and 0.80 ml/minute.
Sample flow = 0.23 and 0.42 ml/minute.
NaoH (1M) flow = 0.10 and 0.16 ml/minute.
HCl (0.01M) flow = 0.23, 0.42, 0.60 and 0.80 ml/minute.

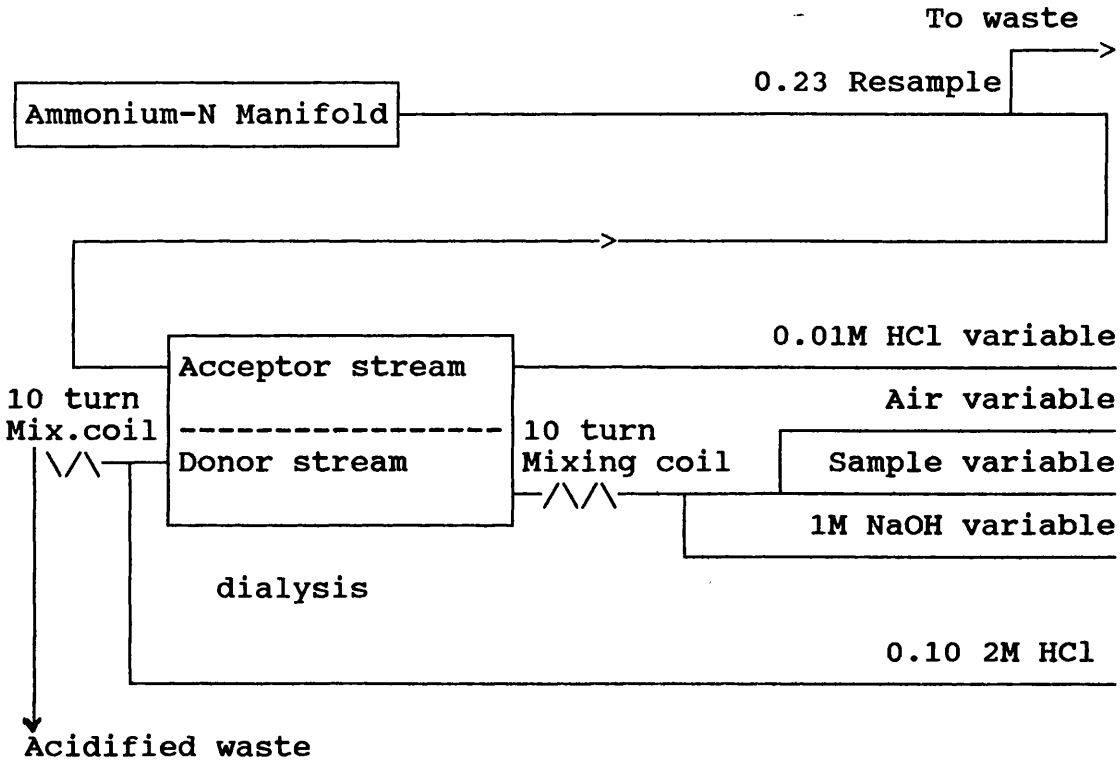

Figure 3.2 Dialysis system 2.

Table 3.11 Effect of flow rate on ammonium-N measurement.

Air Flow (ml)	Sample Flow (ml)	NaOH Flow (ml)	NaOH Conc. (M)	Total Donor (ml)	Acceptor Flow (ml)	Peak Height
0.60	0.23	0.10	1M	0.93	0.32	23.0
0.80	0.23	0.10	1M	1.13	0.32	23.5
0.32	0.23	0.10	1M	0.65	0.32	26.5
0.32	0.32	0.10	1M	0.74	0.42	11.5
0.80	0.32	0.10	1M	1.22	0.42	13.0
0.32	0.42	0.16	1M	0.90	0.60	3.5
0.80	0.42	0.16	1M	1.38	0.60	4.0
0.32	0.23	0.10	1M	0.65	0.60	3.5
0.32	0.32	0.16	1M	0.80	0.80	1.0
0.32	0.42	0.16	1M	0.90	0.42	9.0
0.32	0.42	0.16	1M	0.90	0.32	24.0
0.32	0.42	0.16	1M	0.90	0.23	51.5
0.32	0.23	0.10	1M	0.65	0.42	13.5
0.32	0.23	0.10	1M	0.65	0.32	24.0
0.32	0.23	0.10	1M	0.65	0.23	39.5
0.32	0.42	0.16	1M	0.90	0.23	50.5

The number of air bubbles and their surface area might have impact on the percentage recovery of ammonia through the membrane. The effect of air flow rates is evident from the Table 3.12.

Table 3.12 Effect of air flow at various acceptor flow rates on ammonium measurement.

Air flow rate (ml/minute)	Acceptor flow rate (ml/minute) 0.32	0.42	0.60
0.32	26.5*	13.0	4.0
0.60	23.0	-	-
0.80	23.5	11.5	3.5

* Peak heights

There is small increase in ammonium recovery as air was reduced but is not equivalent to the change in the donor system residential time. So, air flow rate is not an important parameter affecting the recovery of ammonium-N.

It was assumed that the sample flow rate may affect the ammonium recovery. Some data was selected from the Table 3.11 and presented in Table 3.13 to show the relationship between the donor and the acceptor flow rates. The final ammonium concentration of the donor solution (sample plus sodium hydroxide) was equal in the donor stream but the residential time was different. It is clear from the peak heights that faster flow resulted in the greater transfer of ammonia through the membrane, only at low acceptor flow rate.

Table 3.13 Effect of two donor flow rates at various acceptor flow rates on ammonium measurement.

Donor flow rates (ml/minute)	Acceptor flow rates (ml/minute) 0.23	0.32	0.42	0.60
0.65	39.5*	24	13.5	3.5
0.90	50.5	24	9.0	3.5

* Peak heights

The acceptor flow rate was inversely proportion to the peak height as indicated in the Figures 3.3a and 3.3b.

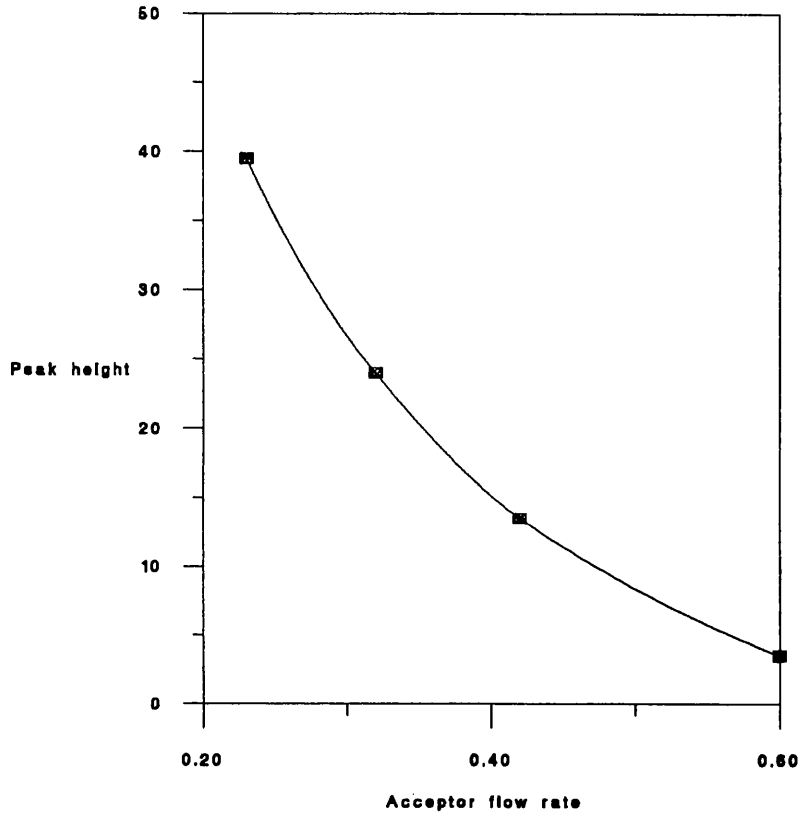

Figure 3.3a Effect of acceptor flow rate on ammonium-N measurement at 0.65 ml/minute donor flow rate

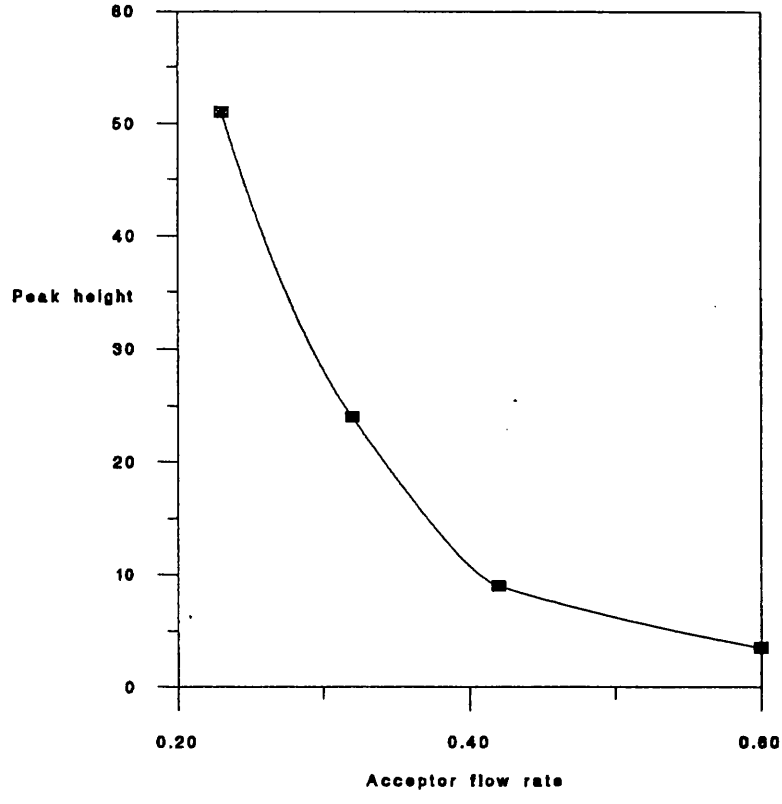

Figure 3.3b Effect of acceptor flow rate on ammonium-N measurement at 0.90 ml/minute donor flow rate

Final dialysis system

The relationship of acceptor flow rate and ammonia trapping was considered a crucial parameter for further work and the best flow rate for donor and acceptor streams was chosen to analyse the water and extracts. This lead to the system shown in the Figure 3.4. In this system, instead of pumping the acid acceptor stream and the resampling, the 0.23 sample line of ammonium main manifold was pulling 0.01M hydrochloric acid through acceptor side of the dialysis.

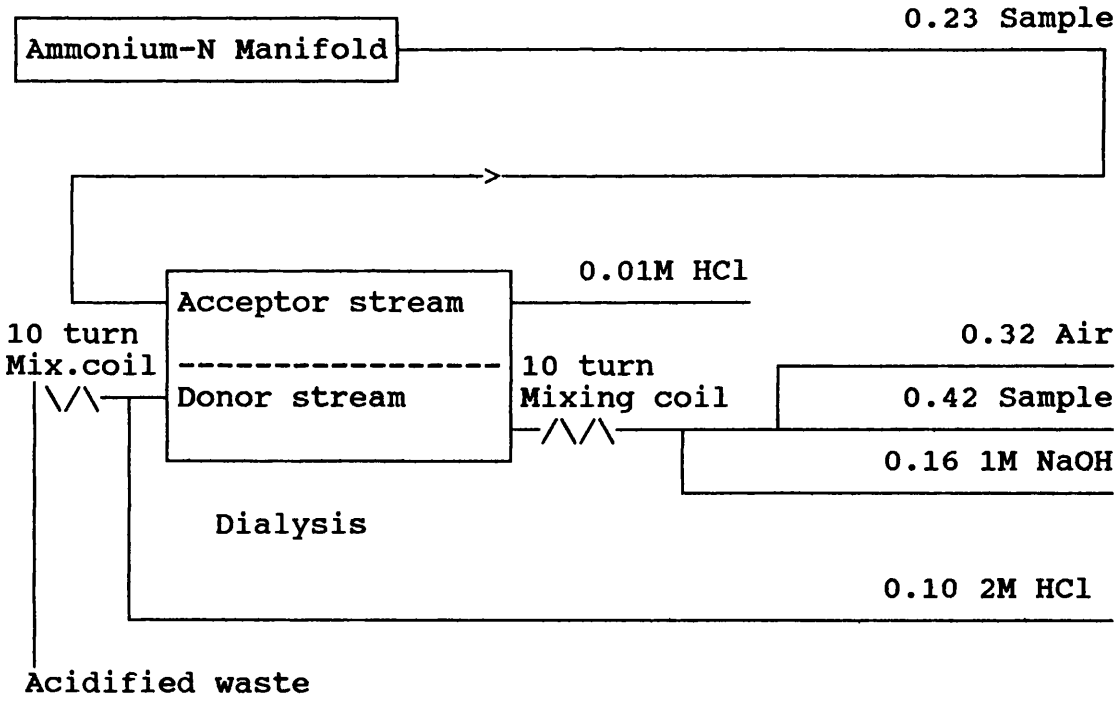

Figure 3.4 Final dialysis system.

3.6.3 Effect of Membranes on Ammonium-N Recovery

Dialysis membranes were tested using the system shown in Figure 3.4. Three and six inches dialyzers with path lengths of 15 cm and 30 cm were tested for using three dialysis membranes. These membranes were the Technicon silicon rubber gas dialysis membrane, PTFE and TFE hydrophobic membranes. The Table 3.14 show the percent recovery of ammonium compared with ammonium measured bypassing the dialysis step.

Table 3.14 Ammonium-N recovery through membranes.

	Dialyzers	
	3 inch	6 inch
Membranes	Ammonium-N recovery (%)	
Silicon	4.2	-
PTFE	59.6	86.8
TFE	53.9	81.5

Technicon silicon membrane showed very low ammonia transference. The hydrophobic membranes allowed similar amounts of ammonia to be transferred through them. Double ammonia transference was expected through 6 inch dialysis but approximately 85% recovery was achieved.

3.6.4 Effect of Samples Rate per Hour on Peak Separation

Sample rates per hour of 50, 40, 30 and 20 were tested. Analysis rate at 20 samples per hour showed improved peak separation. The peak shapes of different samples rate are shown in the Figure 3.5.

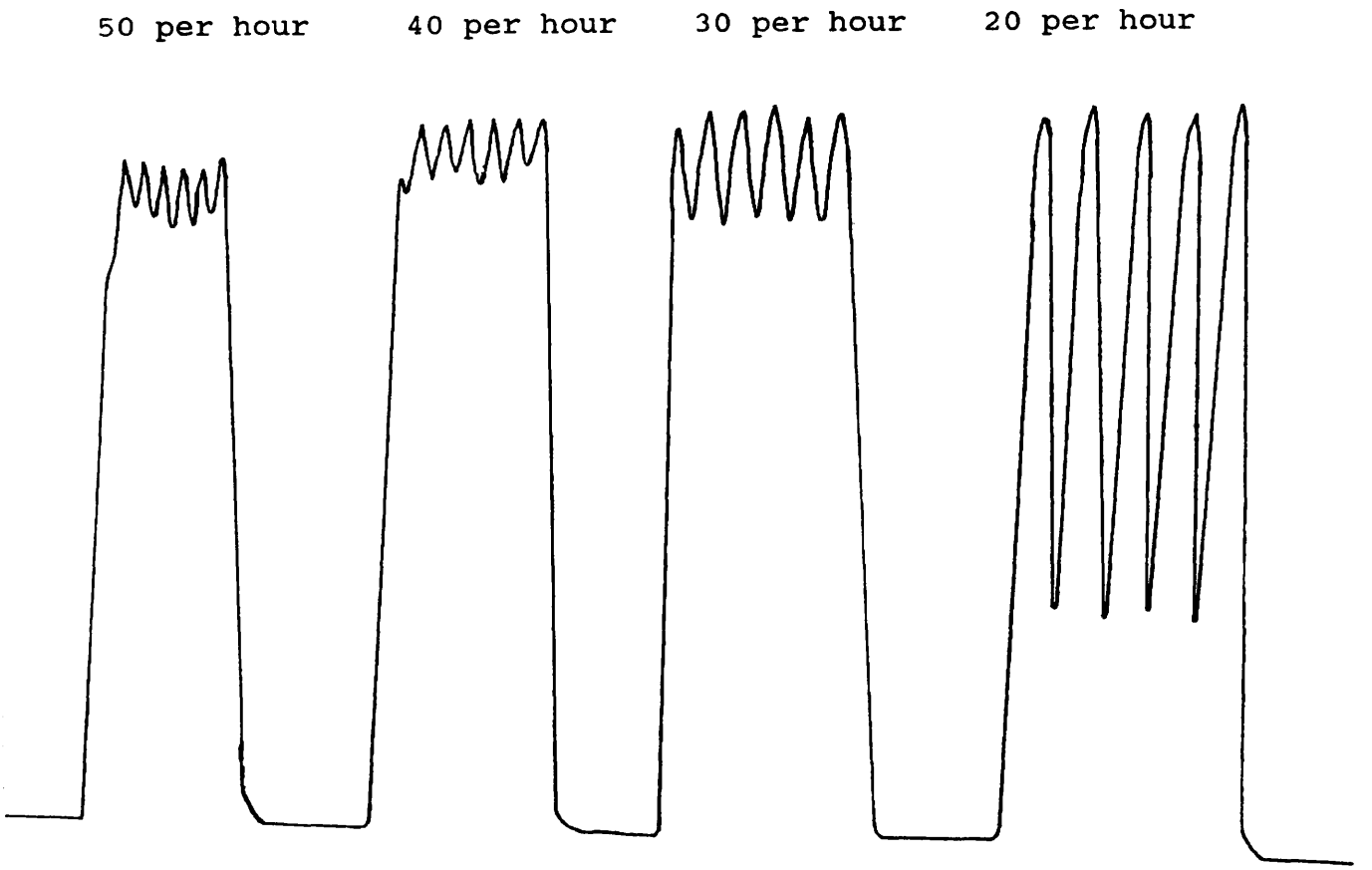

Figure 3.5 Effect of the sampling rate on peak shape

3.6.5 Precision of the Final Dialysis System

Ammonium-N working solution (1 mg l^{-1}) was analysed using the system shown in Figure 3.4 to investigate the precision of the ammonium measurement using the dialysis system with the Technicon AutoAnalyzer II method. Twelve samples of the same solution were analysed in individual sample cups. The results are shown in Table 3.15.

Table 3.15 NH_4-N recovery of same solution

Replicate Number	NH_4-N (mg l^{-1})
1	0.997
2	0.997
3	1.001
4	1.004
5	1.006
6	1.008
7	1.014
8	1.020
9	1.006
10	1.003
11	1.008
12	1.010
Mean	1.006
Standard Deviation	0.0066

The standard deviation of the results shown in Table 3.15 is very low which suggests that the developed method is sufficiently precise and reliable for its use in the ammonium-N determination. Mazumder (1992) examined the ammonium-N recovery in the standard solution prepared in water (1 mg l^{-1}) in ten replicates using the Technicon AutoAnalyzer II without dialysis. The standard deviation of the results was between 0.002 and 0.004 for a 1 mg l^{-1} NH_4-N solution. Comparison of the present results with those of Mazumder (1992) show that the present results are slightly less precise which is to be expected with the more complex system.

3.6.6 Linearity of the Final Dialysis System

To obtain standard calibration curves to investigate the linearity of the system, ammonium solutions (0.02 to 0.1 mg l^{-1}) and (0.2 to 1.0 mg l^{-1}) were analysed using the final dialysis system. The calibration curves are shown in Figures 3.6a and 3.6b. Both calibration curves are linear.

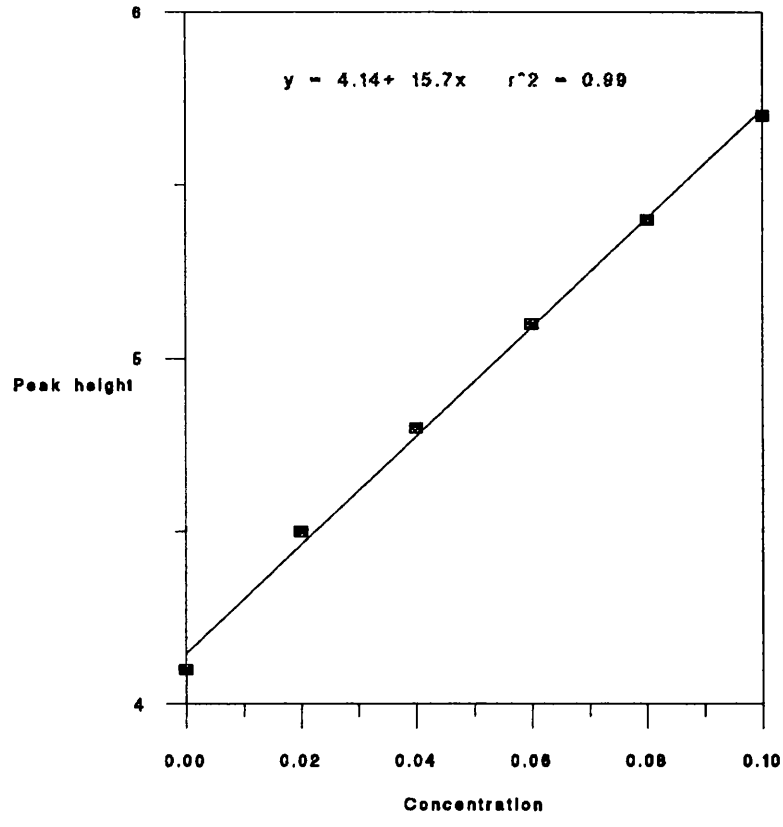

Figure 3.6a Standard curve 0 to 0.1 mg N l-1

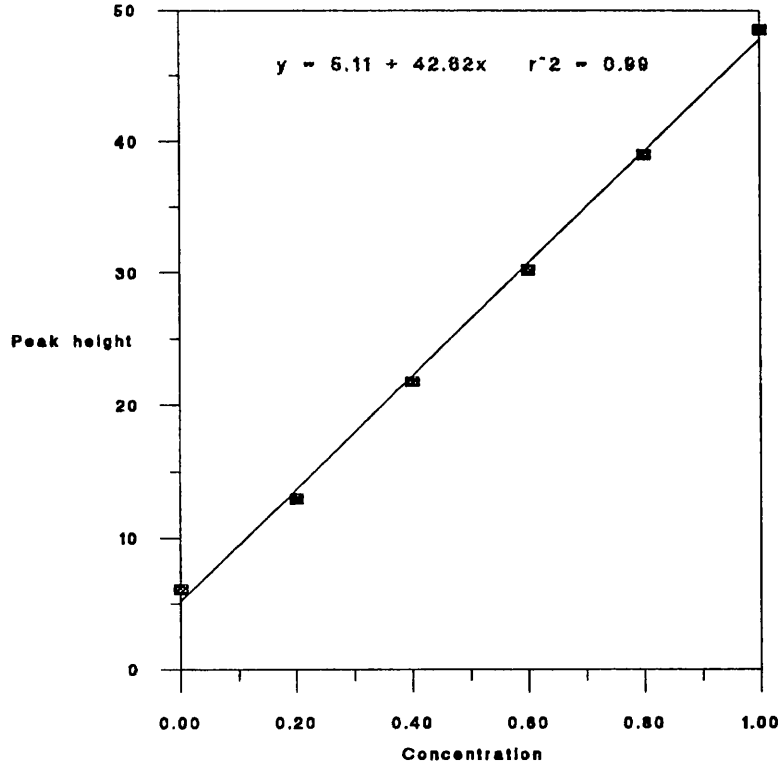

Figure 3.6b Standard curve 0 to 1.0 mg N l-1

3.6.7 Interferences caused by Organic Nitrogen Compounds

Twenty organic compounds were evaluated for their interference potential in the determination of ammonium-N using the final dialysis system with the Technicon AutoAnalyzer II. The results are shown in Table 3.16.

Table 3.16 Interferences by organic nitrogen compounds in water.

Organic compounds	Effect in (mg l^{-1}) N of organic compounds at an ammonium concentration of			
	Without dialysis		With dialysis	
	0	1	0	1
	--(mg l^{-1})--		--(mg l^{-1})--	
Glycine	0.03*	-0.09**	0.00	+0.02
Alanine	0.02	-0.07	0.00	+0.01
Valine	0.00	-0.03	0.00	+0.01
Leucine	0.01	0.00	0.00	-0.03
Isoleucine	0.00	-0.03	0.00	-0.01
Serine	0.02	-0.05	0.00	0.00
Threonine	0.00	-0.08	0.00	0.00
Methionine	0.00	-0.23	0.00	0.00
Aspartic acid	0.04	0.00	0.02	-0.02
Glutamic acid	0.03	-0.05	0.00	0.00
Asparagine	0.01	-0.01	0.00	+0.01
Glutamine	0.02	0.00	0.10	-0.04
Lysine	0.02	-0.04	0.01	-0.02
Arginine	0.00	-0.01	0.00	-0.02
Phenylalanine	0.00	-0.12	0.00	+0.01
Proline	0.00	-0.07	0.00	0.00
Histidine	0.00	-0.03	0.01	-0.02
Urea	0.02	+0.02	0.02	-0.03
Galactosamine	0.17	-0.01	0.03	+0.01
Glucosamine	0.02	-0.02	0.00	+0.01

* Each value is the mean of two replicates.

** This is a corrected value after deducting the interference (mean of two replicates) caused by each amino acid at 0 mg l^{-1} N.

Columns 1 and 2 of Table 3.16 which show the interferences caused by the organic nitrogen compounds in water without dialysis are brought from Table 3.7. The results in column 3 and 4 show the chemical interferences caused by the organic nitrogen compounds in ammonium-N determination using the dialysis system with the Technicon AutoAnalyzer II.

If there were ammonium-N impurities in the organic nitrogen compounds, they would show positive interferences in the ammonium-N measurement even with dialysis. But the dialysis results (column 3) show that the chemical interferences caused by organic compounds (without dialysis) as shown in column 1 were mostly removed or were much reduced after inclusion of the dialysis step in the system. Small effects shown by aspartic acid, lysine and histidine may be due to trace ammonium impurity. Only urea showed clear evidence of the presence of ammonium-N impurity as its positive interference remained unchanged. The effects in column 1 are not, therefore, due to ammonium-N impurities, but are probably due to the hydrolysis of the organic nitrogen compounds or their taking part in the Berthelot reaction. Glutamine gave an odd result with the dialysis system. There was low possibility of glutamine hydrolysis because the pH of the final mixture solution in the donor stream was 12.0. Its greater positive interference might be due to some ammonium-N cross contamination during the sample preparation at this time of analysis.

The addition of organic nitrogen compounds showed very small effects on the ammonium-N measurement at 1.0 mg l^{-1} ammonium-N. The chemical interferences were mostly reduced with the inclusion of the dialysis in the ammonium system of the Technicon AutoAnalyzer II. For example, the negative interferences by methionine and phenylalanine were reduced from 23% and 12% to 0% and 1%, respectively. The small effects shown in the column 4 on the ammonium-N recovery showed both positive and negative deviations. These small effects are probably not chemical interferences but are due to the random error caused by the solution variability and system complexity. Evidence for this view can be obtain from the precision study (see, Table 3.15). This study showed a small level of variability in the ammonium-N measurement of the same solution of approximately the same magnitude as in column 4 of Table 3.16.

The following points can be concluded from the above discussion:-

Only traces of ammonium-N were present as impurities in some organic nitrogen compounds such as urea, aspartic acid, lysine and histidine.

Positive and negative interferences were found due to hydrolysis/participation of organic nitrogen compounds in the absence of the dialysis in the ammonium-N system of the Tecnicon AutoAnalyzer II method.

Positive and negative interferences were eliminated by dialysis.

Errors with dialysis were consistent with random variability of solution preparation and to the system complexity.

Ammonium-N can be determined in the presence of the organic nitrogen compounds in samples if dialysis is included as a pre-treatment in the ammonium-N system of the Technicon AutoAnalyzer II.

3.6.8 Evaluation of the Dialysis System analysing Kjeldahl digsts.

Kjeldahl digests contain high acid concentration and high ammonium-N content. These digests can be diluted and neutralized with sodium hydroxide before the determination of ammonium-N by the Berthelot reaction. However, the digests vary in their acid content due to acid evaporation during the digestion and reaction of acid with the sample. Therefore, even after the dilution of the digests with deionised water, still there is variation in the final concentration of the acid of the samples. This acidity variation of the samples could cause variation in the pH of the final mixture reaction during the colour development. Consequently, it will affect the colour formation among the samples. It was, therefore, appropriate to test the developed dialysis system shown in the Figure 3.4 for the analysis of the Kjeldahl digests to eliminate the acidity effect on the colour development during the Berthelot colour reaction.

Factors like dilution and neutralization of the digests, temperature rise due to the reaction of acid and sodium hydroxide and the donor flow rate may have

influence on the ammonia transfer through the hydrophobic membranes used in the dialysis. Therefore, the system shown in the Figure 3.4 was subjected to different modifications for its applicability for Kjeldahl digests. The initial objective of these changes was to perform the dilution, neutralization and dialysis in a single step but this was not achieved because of unstable flow through the donor stream of the dialysis system. So, a separate dilution step was included with the dialysis system and the neutralization and dialysis were performed in the second step as shown in the Figure 3.7.

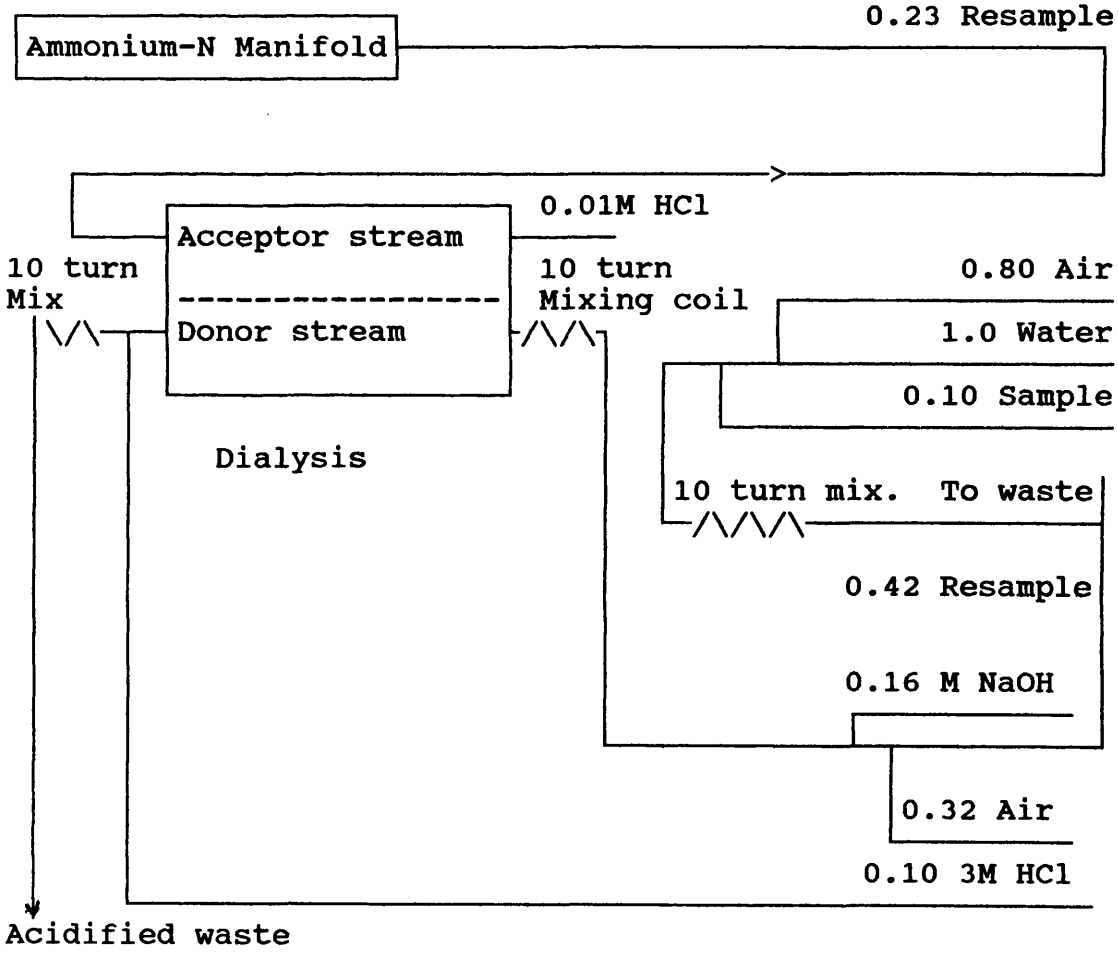

Figure 3.7 Dialysis system for total nitrogen measurement.

Fifty Kjeldahl digests of grass were analysed for their ammonium-N content with and without dialysis. The results of both methods are shown in the Figure 3.8.

It is evident from these results that the two methods showed very good correlation. Although a paired T test showed a significant difference ($p<0.001$) between the two methods, the means differ only by 0.05 %. It appears that the acid variation in the digests did not show adverse effect on the determination when using simple dilution.

Precision of the Final Dialysis System

The same Kjeldahl digest was analysed in 20 individual sample cups with and without the dialyses. The mean and the standard deviation are shown in Table 3.16.

Table 3.16 Precision of the dialyses system.

	Without dialysis	With dialysis
Mean	2.84	2.77
Standard Deviation	0.0230	0.0153

The above Table shows that both methods used for ammonium-N determination in Kjeldahl digest gave very low standard deviations. Therefore, dialysis use with the Technicon AutoAnalyzer II did not disturb the precision of the system.

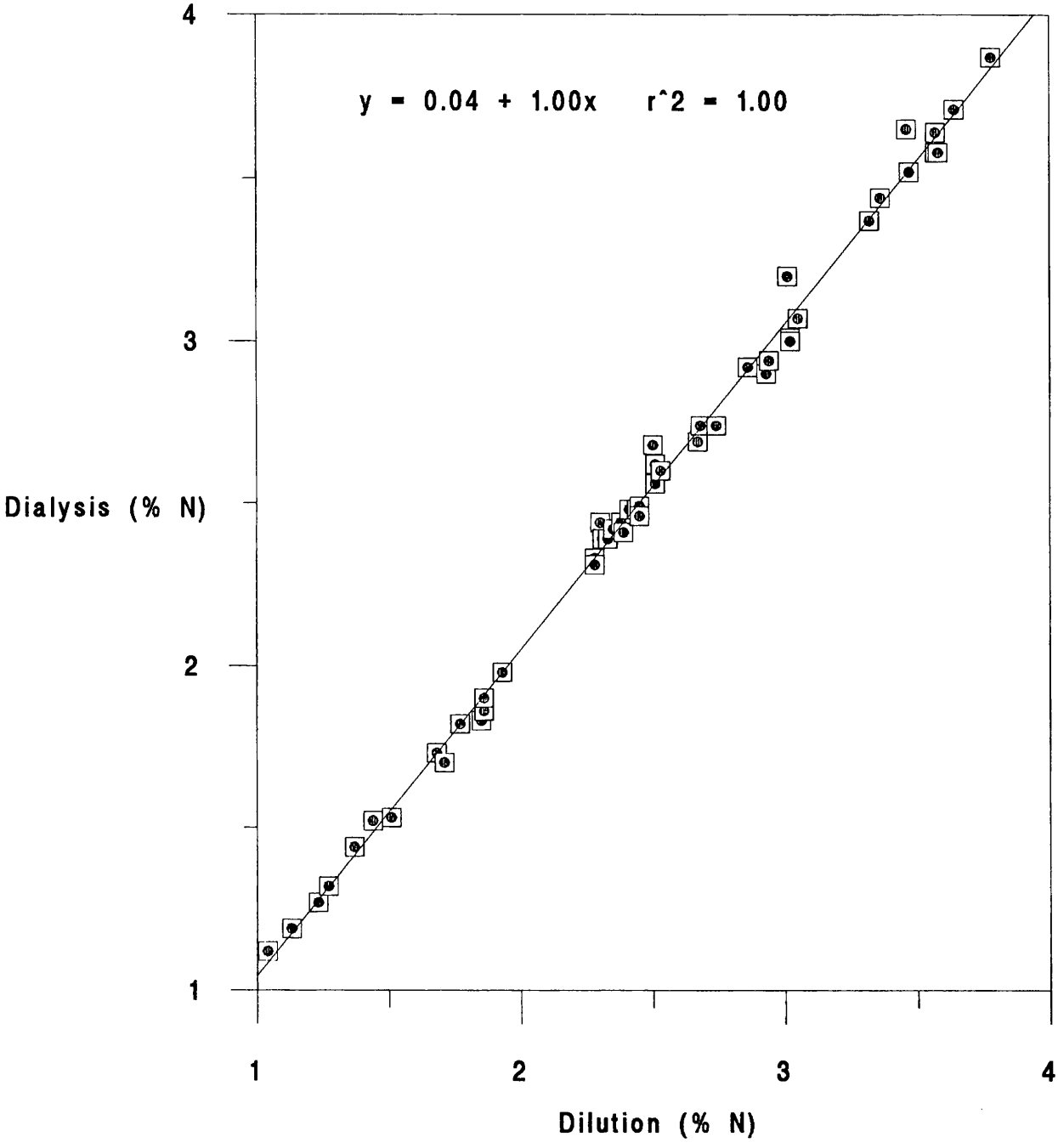

Figure 3.8 Comparison of total nitrogen content of grass samples measured by dialysis and dilution

CHAPTER FOUR

PLANT AVAILABLE NUTRIENTS IN ALGAL POND EFFLUENT

4.1 INTRODUCTION

Problems of the disposal of agricultural effluents most frequently concern pig slurry because herd size need not to be related to the area of land available for disposal. The search for alternative disposal systems has been stimulated by the cost of disposal, concern over environmental effects and possible soil and plant nutrition problems associated with heavy or inappropriately timed slurry spreading.

Aerobic treatment whilst lowering the organic matter content and offering a method to control odour still results in an effluent rich in nitrogen and phosphorus which may restrict its disposal to land during certain times of the year (Groeneweg and Soeder, 1985). The further removal of phosphorus and nitrogen would reduce the potential for eutrophication, and through treatment of the liquid phase, reduce the volume for land disposal. Such a strategy may also be applicable to the treatment of many other wastes such as sewage and waste waters. Controlled growth of algae in a High-Rate Algal Pond (HRAP) may be one method by which this strategy may be achieved. Fallowfield and Garrett (1985) demonstrated that controlled algal growth in separated, diluted pig slurry liquid phase resulted in high nitrogen and phosphorus removal rates. Svoboda and Fallowfield (1989)

established the aerobic treatment plant for the pig slurry at Scottish Agricultural College, Auchincruive, Ayr. The reactor (3.4m in diameter and 2.65m high) was made from glass coated steel panels on a concrete base. Temperature of mixed liquor in the reactor was controlled by heat extraction with a stainless steel tubular heat exchanger (100m of 22mm tube) connected to a 12 kW heat pump, which upgrades and transfers this heat into a weaner house heating circuit with maximum water temperature of 55°C. The aerobically treated slurry was stored in settling tanks. The effluent was used following dilution as a substrate for an algal culture. Settled sludge was discharged to the farm slurry storage tank for subsequent field spraying. They built two concrete High-Rate Algal Ponds, each of 13 m^2 and containing 2,600 liters at 20 cm depth. The raceways were mixed by twelve bladed paddlewheels powered by variable speed electric motors, at a mean surface velocity of 21cm/s. When not required for space heating in the weaner house the warm water was redirected to a stainless steel heat exchanger in one of the algal raceway to elevate its temperature. The Other algal raceway was operated at ambient temperature thus permitting a comparison of the effects of intermittent heating upon algal productivity and effluent treatment.

Settled, diluted (1:4) liquid phase of aerobic reactor effluent was pumped to the raceway by either a peristaltic or diaphragm metering pump. The rate of liquid phase addition controls the retention time.

Constant culture volume was maintained by an upright overflow pipe. The reduction in the initial nutrient level of sedimented pig slurry liquid phase was monitored following algal culture at ambient and elevated temperatures. The highest mean percentage nitrogen removal was recorded in August in the ambient temperature pond. Nitrification was evident in both HRAP's in September and October. Percentage removals of phosphorus and nitrogen were lower in both HRAP's than those recorded in an earlier study (Fallowfield and Garrett, 1985) for separated, diluted (1:10) pig slurry liquid phase without prior aerobic treatment.

Fallowfield (1987) reported that HRAP's, where algal photosynthesis is optimised, are more efficient than waste stabilization ponds in terms of land area and retention times required to achieve satisfactory effluent. He suggested that use of algae, in suitable climatic conditions to treat effluent is the most immediately exploitable form of algal biotechnology. HRAP's are extremly efficient biomass production system. This biomass can be used as a green fertilizer.

Plaixats et al. (1988) analysed piggery waste, effluent residues and supernatant for their properties and chemical composition. Large quantities of mineralizable or available nutrients were found in the effluent after anaerobic digestion. They recommended that the effluents are better for agricultural use as fertilizer than undigested pig excreta.

Walker and Younos (1987) studied a laboratory-scale aquaculture system for treatment of diluted manure. They selected dairy waste because it had the greatest threat to Chespeake Bay (USA) water quality. Duckweed was selected because of its suitable characteristics, such as its tolerance of a wide range of temperature and its potential use for high protein livestock feed. The laboratory waste treatment systems consisted of two groups of three small pools connected in series. One group contained duckweed, while the other served as a control. Based on the collected data, at optimum aquatic plant performance, duckweed pool water contained 43% less total Kjeldahl nitrogen and 35% less total phosphorus than the control. Optimum performance was estimated to occur at a manure concentration of one part manure to 100 parts manure and water.

Small scale, 2 m^2 surface area, High-Rate Algal Ponds were being run by the Department of Biochemistry of the Scottish Agricultural College under controlled conditions in the greenhouse at Auchincruive, Ayr (Fallowfield, unpublished). The ponds received a steady input of a synthetic pig slurry. The feed consisted of an organic and an inorganic fraction. The organic fraction was sodium acetate as a source of carbon. A liquid N, P and K fertilizer in the ratio of 16:6:9 containing nitrogen as urea was fed into the pond as an inorganic fraction. The trace metals essential for algal and bacterial growth were also added. The pond solution was constantly mixed to keep the algae and bacteria suspended

and in an adequate light environment. The growth of the bacteria and algae results in the transformation of the organic and inorganic pond fractions into more stable algal and bacterial cells. An important aspect of waste treatment is that soluble and potentially polluting inorganic nutrients are immobilised in the biomass.

The studies were being carried out to see the effect of changes in the loading of carbon and nitrogen in the pond effluent on algal and bacterial growth. The treated effluent overflowed and collected in a holding tank. There was an intention to dispose of this waste to agricultural land. It was, therefore, important to investigate the composition of the algal pond effluent in relation to its ability to supply plant available nutrients. The samples were obtained from these small scale ponds because large ponds (13 m^2) were not being used at that time by the department.

Selection of a suitable extracting solution for the determination of the available nutrients in animal wastes depends upon the nutrients to be determined in the extracts and also on the instrument used in their determination. Various extracting solutions have been used for extraction of the available nutrients from animal wastes. Hoyle and Mattingly (1954) have used dilute hydrochloric acid (0.1M HCl) to extract soluble nitrogen from compost. They extracted soluble nitrogen at the ratio 1:10 fresh compost:0.1M HCl. Byrne and Power (1974) suggested that ammonium nitrogen can be extracted with dilute 0.1M HCl from animal slurries. Ammonium

nitrogen is closely related to the soluble nitrogen content in animal slurries which may either be used to give an estimate of the total nitrogen content or could be used to indicate the efficiency of the slurry relative to fertilizer nitrogen. Their method of extraction involved 10 g slurry plus 90 ml 0.1M HCl. The suspension was shaken for 1 hour. Diaz-Fierros et al. (1987) used 0.5M acetic acid as an extractant for the extraction of the physical fractions of cattle slurry. They determined phosphorus, potassium, calcium, magnesium, sodium, nitrate-N and ammonium-N in the extract. Kirchmann (1990) used 0.27M, 1.75M and 12.5M sulphuric acid in the extraction studies on poultry excrement labelled with ^{15}N. Rowarth et al. (1985) determined extractable phosphorus in the extracts obtained by shaking of 1 g of sheep dung with 40 ml of the distilled water. The samples were shaken for six hours and were then centrifuged and filtered.

Rixon and Zorin (1978) used potassium chloride solution as an extractant for determination of mineral nitrogen content of faecal material. Inorganic phosphorus was extracted by shaking of faecal material in 2% sulphuric acid solution and filtering. Xie and MacKenzie (1986) determined the ammonium-N in 1M potssium chloride extracts of fresh and composted cow manure and liquid hog manure. Similarly, ammonium-N was measured in 2M potassium chloride solution extracts of liquid poultry manure, liquid cattle manure and solid beef cattle manure (Beauchamp, 1986). Whitehead et al. (1989) extracted the

ammonium-N from cattle and pig slurries with 1M potassium chloride. They analysed the extracts for ammonium-N by automated colorimetry involving the Berthelot reaction. Mahimairaja et al.(1990) also reported that inorganic forms of nitrogen were measured by extracting samples of fresh poultry manure, composted poultry manure, sheep manure, horse manure, dairy manure, dairy slurry and pig slurry in 2M potassium chloride for 30 minutes at a solid:solution ratio 1:10. Inorganic forms of nitrogen in potassium chloride extract were measured by following the nitroprusside catalysed Berthelot reaction for ammonium-N and a diazotization coupling reaction method for nitrite-N and nitrate-N. For the measurement of nitrite-N alone, hydrazine which reduces nitrate to nitrite was not added. Recently, Paul and Beauchamp (1993) determined the ammonium-N and nitrate-N in 2M potassium chloride extracts of liquid dairy cattle manure, solid beef cattle manure and composted beef cattle manure.

Keeping in view the difference in the extractants used in farm wastes studies, it was imperative to conduct a study of the measurement and extraction of nitrogen, phosphorus and potassium from algal pond effluent comparing 0.1M hydrochloric acid, 0.05M sulphuric acid, 0.05M sodium sulphate and water as extractants alongwith a direct filtration of effluent. The selection of a suitable extractant for measuring available nutrients in the algal pond effluent was the first objective of this study. The second aim was to see the effect of storage on nutrient stability in the algal pond effluent.

4.2 METHODS AND MATERIALS

4.2.1 Algal Pond Effluent Samples

The samples were collected from the small scale experimental algal ponds from the greenhouse at Scottish Agricultural College, Auchincruive, Ayr. The samples were stored in the cold room at 2°C until required.

4.2.2 Determination of Dry Matter Content

Glass fibre filters grade C were washed with deionized water and dried overnight in an oven at 110°C. Each filter was weighed and used in the filtration. 500 ml waste was filtered. The used filters were dried at 110°C and the % dry matter of the waste was calculated as follows

$$\% \text{ Dry matter} = \frac{\text{weight of dry matter}}{\text{volume of sample filtered}} \times 100$$

4.2.3 Extraction of Macronutrients from Algal Pond Effluent

4.2.3.1 Reagents

0.1M hydrochloric acid

8.5 ml concentrated HCl was diluted to 1 litre with deionized water carefully in the fume cupboard.

0.05M sulphuric acid

2.75 ml concentrated sulphuric acid was added to approximately 500 ml deionized water. When cooled, volume was made to 1 litre.

0.05M sodium sulphate

15.2 g sodium sulphate was dissolved in about 800 ml deionized water and volume was made up to 1 litre. The solution was purified of ammonium nitrogen contamination by raising its pH to 11.0 with 1M sodium hydroxide solution. It was then boiled for 15 minutes to give off ammonia gas. The solution was allowed to cool and the pH was readjusted to pH 6.0 with 0.5M sulphuric acid. Deionized water was added to allow for any loss of water during the boiling of the solution due to evaporation (Khan, 1987).

4.2.3.2 Washing of filter papers

Each filter paper was folded in a clean and dry plastic funnel. It was washed with 50 ml of 0.5M sulphuric acid by filtration in two equal portions of 25 ml each. Then these were rinsed five times with deionized water to wash away any acid deposit. Universal pH paper was used as a check to be certain that filter papers were acid free. The washed filter papers in the funnels were dried for four hours in the oven at 70°C before use (Shah, 1988).

4.2.3.3 Extraction procedure for algal pond effluent

The algal pond effluent was extracted with four extractants which were 0.1M hydrochloric acid, 0.05M sulphuric acid, 0.05M sodium sulphate and water alongwith direct filtrate of the effluent. The aim of this experiment was to select a suitable extractant for the

extraction of the available nitrogen, phosphorus and potassium from the algal pond effluent.

The stock of algal pond effluent was stirred using a magnetic stirrer. 10.0 g of algal pond effluent was weighed into 100 ml plastic bottles using four replications and blanks for each treatment. 90 ml of each extractant was added to these bottles. The blank bottles contained 100 ml of the extractants. The bottles were tightly capped and were shaken for 1 hour on an end-over-end shaker at room temperature. The solutions were then filtered through washed Whatman No. 1 filter papers. The filtrate was collected into 100 ml plastic bottles and stored at 2°C until analysis.

4.2.3.4 Direct filtration

500 ml of algal pond effluent was filtered through a Glass Fibre grade C filter paper in four replications. These were first washed with deionized water. The first 250 ml of sample was discarded as a filter wash and the remaining 250 ml was collected and stored in the fridge until analysis. Appropriate blanks were also included.

4.2.3.5 Experimental design

The algal pond effluent was extracted and filtered using 0.1M hydrochloric acid, 0.05 sulphuric acid, 0.05M sodium sulphate, water and direct filtrate as five treatments. The experiment was conducted using four replications.

4.2.4 Determination of Macronutrients Ions

Ammonium, nitrate, nitrite and phosphate were determined by using the Technicon AutoAnalyzer II (as in section 2.5.1, 2.5.2 and 2.5.3). Potassium was measured by using a flame photometer (as in section 2.5.4).

4.2.4.1 Ammonium-N determination

Ammonium standard stock solution (1000 mg l^{-1})

1.1791 g of ammonium sulphate analar grade and dried at 110°C was dissolved in 250 ml deionized water. The stock solution was stored at 2°C.

Ammonium working standard solution (100 mg l^{-1})

10 ml of standard stock solution (1000 mg l^{-1}) was diluted to 100 ml with deionized water in the volumetric flask.

Ammonium determination in extracts

0 and 1 mg l^{-1} ammonium nitrogen working standard solutions were prepared by pipetting 0 and 1 ml of working solution into 100 ml flasks and the volume was made with each extractant. The extracts were analysed without dilution at a sampling rate of 50 per hour.

Ammonium determination in direct filtrate

The ammonium nitrogen working standard solutions from 0 to 20 mg l^{-1} were prepared in deionized water. The direct filtrates were diluted in the ratio of 0.1:2.0

sample:deionized water by an inbuilt diluter. The filtrate was analysed at 40 samples per hour.

4 2.4.2 Nitrate-N determination

Nitrate standard stock solution (1000 mg l^{-1})

1.8046 g of potassium nitrate was weighed on four figure balance and transferred to 250 ml volumetric flask. It was dissolved in deionized water and made to volume. This solution was stored at 2°C.

Nitrate working standard solution (100 mg l^{-1})

10 ml of the 1000 mg l^{-1} standard stock solution was pipetted into a 100 ml flask and the volume made to the mark with deionized water.

Nitrate determination in extracts

0 and 20 mg l^{-1} nitrate nitrogen working standards were prepared in each extractant. The extracts and standard were diluted in the ratio of 0.1:2.00 by an inbuilt diluter and sample analysis rate was 40 per hour.

Nitrate determination in filtrate

The nitrate working standard solutions 0 and 1 mg l^{-1} nitrate were prepared in deionized water. The filtrate was manually diluted in the ratio of 1:100 samples:deionized water. The samples were analysed at a rate of 40 per hour.

4.2.4.3 Phosphate-P determination

Phosphate standard stock solution (1000 mg l^{-1})

1.0983 g potassium dihydrogen phosphate was weighed and dissolved in 250 ml deionized water to give 1000 mg l^{-1} of phosphate solution. It was stored at 2°C.

Phosphate working standard solution (100 mg l^{-1})

10 ml of the phosphate standard stock solution was diluted to 100 ml in deionised water.

Phosphate determination in extracts

0 and 5 mg l^{-1} phosphate working standards were prepared in appropriate extractants. The extracts were analysed without dilution and the sample rate per hour was 40.

Phosphate determination in filtrate

Phosphate was measured in the filtrate with 0 and 20 mg l^{-1} phosphate working standard solutions in deionized water. The dilution was 0.1:2.00 sample:deionized water by an inbuilt diluter. Samples were analysed at a rate of 40 per hour.

4.2.4.4 Potassium determination

Potassium standard stock solution (1000 mg l^{-1})

0.4766 g potassium chloride was dissolved in deionized water in 250 ml volumetric flask. It was stored at 2°C.

Potassium working standard solution (100 mg l^{-1})

10 ml of standard stock solution was pipetted into a 100 ml flask and volume was made with deionized water.

Potassium determination in extracts

0, 2, 4, 6 and 8 mg l^{-1} potassium working standards were prepared in each extractant and the flame photometer was calibrated using 0 to 8 mg l^{-1} standards.

Potassium determination in filtrate

Potassium working standard solutions 0, 2, 4, 6 and 8 mg l^{-1} potassium were prepared in deionized water and used in the calibration of the flame photometer. The samples were diluted manually in ratio 10:100 sample:deionized water.

4.2.5 Nutrients Stability in Algal Pond Effluent

The stock of algal pond effluent in a five litre container was stored at 2°C in the cold room. The required portion out of it was brought into the laboratory weekly, filtered and analysed for plant available nutrients. A 0.1M HCl extract of this effluent was also obtained at the same time. Nitrite and nitrate were determined from the filtrate while the ammonium, phosphate and potassium were measured in 0.1M HCl extracts. The objective of this study was the measurement of any possible change in the chemical composition of algal pond effluent during storage.

Table 4.1 Analytical conditions for nutrient stability experiment.

Nutrients	Dilution (ml)	Standard (mg l^{1})	Samples/ (Hour)	Filtration/ Extraction
NH_4^+-N	1:10	0-10	40	0.1M HCl
NO_2^--N	1:50	0-1	40	Filtrate
NO_3^--N	1:50	0-1	40	Filtrate
PO_4^{3-}-P	none	0-5	40	0.1M HCl
K	-	0-8	-	0.1M HCl

4.2.6 Results and Discussion

Extraction of macronutrients from algal pond effluent

The results obtained are shown in the Table 4.2. This table shows that 0.1M HCl extracted significantly more available ammonium-nitrogen than the other extracts. It did not differ significantly from the ammonium measured in the direct filtrate. No difference in the nitrate level extracted by 0.1M hydrochloric acid and 0.05M sulphuric acid was observed. However, the direct filtrate gave a significantly lower amount of nitrate compared with all the extracts. Either extract or direct filtrate can be analysed for the measurement of available nitrogen.

Table. 4.2 Available nutrients in extracts and direct filtrate of algal pond effluent.

Extractant/Filtrate	Nutrients			
	NH_4^+-N	NO_3^--N	PO_4^{3-}-P	K
0.1M HCl	11.4c	76.9bc	13.7cd	60.4b
0.05M H_2SO_4	10.3a	76.5b	13.9d	61.7c
0.05M Na_2SO_4	10.7b	78.0d	12.2a	58.1a
Deionized water	10.6b	77.7cd	12.9b	60.5b
Direct filtrate	11.3c	75.0a	13.4c	60.2b

Figures in a column with the same letters following are not significantly different at 5% level using a Fisher's LSD Test.

The available phosphate in extracts and direct filtrate shown in Table 4.2 reveal that 0.1M hydrochloric acid extract and direct filtrate had similar concentration of phosphate. 0.05M sulphuric acid extracted slightly more phosphate. However, it did not differ significantly from the phosphate figure of 13.7 mg l^{-1} found in the 0.1M hydrochloric acid extract. Evidently, 0.1M hydrochloric acid, 0.05M sulphuric acid extraction or direct filtration can be adopted for phosphate determination in the algal pond effluent.

It is clear from the potassium results that 0.05M sodium sulphate significantly was the poorest extractant in extracting available potassium. Direct filtration, water and 0.1M hydrochloric acid extracts show similar potassium concentrations. The 0.05M sulphuric acid

extracted significantly more potassium than the other extractants used.

It can be deduced from these results that extracts of treated effluent with dilute acids or sodium salt solution showed similar levels of ammonium, phosphate and potassium which are not different to levels of ammonium, phosphate and potassium extracted by water. This is evidence of the inability of the effluent to hold these nutrients in the solid fraction. This matter of nutrient binding clearly differs from that found in soils where the nutrient holding is so strong that extraction with water or even a sodium salt solution is unable to remove all ammonium and potassium. Nitrate is free in soil solution and may be extracted with water. Phosphate is very strongly held by soil surfaces, therefore, extracting solutions such as acetic acid or sodium bicarbonate buffer are required for its extraction.

Nitrogen, phosphorus and potassium distribution in liquid and solid portions of animal slurries would also be different to the treated effluent tested here. For example, water soluble ammonium-nitrogen in pig slurries was on average 80% of ammonium-nitrogen extracted by 0.1M hydrochloric acid in an experiment conducted by Byrne and Power (1974). The treated effluent fed by synthetic pig waste has free cations and anions and is a different material from algal pond effluent fed by real animal wastes. Acids or salt solutions will be needed for extraction and measurement of ammonium, phosphate and potassium in algal pond effluent.

The colorimeteric methods of analysis require clean and colourless solutions. The rate of filtration and the colour of the filtrate are two more important considerations in the selection of a suitable extractant. Hoyle and Mattingly (1954) decolourised some extracts with hydrogen peroxide in nitrate measurement by colorimetric estimation with phenoldisulphonic acid. In the present study, acid extracts proved better than 0.05M sodium sulphate and water due to their flocculating nature. As far as chemical interferences during the measurement of ammonium, nitrite, nitrate, and phosphate by colorimetric methods are concerned, the chloride ion interfered with nitrate, however, this problem can be solved by dilution of the extracts with water. Nitrite can not be measured in acid extracts as it is unstable under these conditions. The direct filtrate of the effluent can be analysed for nitrite and nitrate content without any problem.

Keeping in view these results, it was decided that ammonium, phosphate and potassium should be measured in the 0.1M hydrochloric acid extracts while nitrite and nitrate should be measured by direct filtration.

Nutrients stability in algal pond effluent

The results of this experiment are shown in Table 4.3.

Table 4.3 Effect of storage on the chemical composition of algal pond effluent.

Nutrients	Storage (weeks) 0	1	2
Ammonium-N	25.8a	28.8b	29.9b
Nitrite-N	5.5*	6.4	5.3
Nitrate-N	42.6*	49.7	45.7
Phosphate-P	15.3a	14.5b	16.0c
Potassium	59.2a	57.8a	57.8a

Figures in a row with the same letter following are not significantly different at 5 % level using a Fisher's LSD Test.

* Anova could not be applied because of insufficient replicates.

It was difficult to bring fresh samples of algal pond effluent from the Scottish Agricultural College, Auchincruive, Ayr, prior to each application to the pot experiment to be conducted. It was, therefore, desirable to store the treated effluent in the cold room at 2°C before each application. As the treated effluent was to be stored, it was imperative to test nutrient stability during storage.

The results shown in Table 4.3 reveal that there are significant changes in ammonium and phosphate content of the effluent. There are also variations in nitrite and nitrate levels although statistics could not be applied

due to insufficient replications. Potassium levels in the effluent showed no significant change. The trend of change in the chemical composition of algal pond effluent suggests that it can be stored for a week at 2°C with only minor changes in ammonium, nitrite, nitrate, phosphate and potassium during storage. Fresh or stored samples can be applied to the pot experiment but their analysis prior to each application will be essential.

CHAPTER FIVE

ALGAL POND EFFLUENT APPLIED AS FERTILIZER TO RYEGRASS

5.1 INTRODUCTION

Algal pond effluent although a very dilute liquid, contains plant available nutrients and organic matter including algal/bacterial biomass (Chapter 4). Its application to crops as a fertilizer seems worth investigation. Therefore, a pot experiment was designed to evaluate the agronomic value of the effluent applied as a fertilizer to perennial ryegrass. The experiment was carried out under greenhouse conditions. Ryegrass is an easily manageable crop and offers several cuts to the farmers. Its advantages over arable crops lies in its well developed root system and provision of a good soil cover. Hence, it is helpful in preventing losses of nutrients by runoff water and leaching. As it is an efficient crop in utilizing applied nutrients under field and greenhouse conditions it was, therefore, selected as the test crop in this study.

Physically, the algal pond effluent collected from experimental algal ponds at Scottish Agricultural College, Auchincruive, Ayr was a dilute liquid containing suspended solids. It contained approximately 0.2% dry matter. Its chemical characteristics are given in Table 5.1. An important aspect of this study was to investigate effect of the effluent on the nitrogen balance in soils and plants. High levels of effluent would need to be applied

to the pot experiment, and this might cause waterlogging in the pots which could lead to nitrogen losses by denitrification. Routine watering can also create anaerobic conditions during the course of the experiment. A capillary watering system was, therefore, designed to provide adequate water supply and prevent nutrient loss by leaching and denitrification. The algal pond effluent was compared with an untreated control treatment, a standard fertilizer treatment and the synthetic effluent equivalent in the available nutrients level to the algal pond effluent treatment.

Table 5.1 Plant nutrients in algal pond effluent.

Nutrients	Available ($mg\ l^{-1}$)	Total ($mg\ l^{-1}$)
Kjeldahl-N	——	348.0
Ammonium-N	73.6	——
Nitrite-N	0.8	——
Nitrate-N	75.3	——
Phosphate-P	22.6	53.4
Potassium	60.0	61.0

5.2 METHODS AND MATERIALS

5.2.1 Soil Sampling Sites

A brief description of the soil sampling sites is given below. Soils were attributed to soil series using the soil memoirs and soil maps for each area (Grant et al.,1962; Ragg et al., 1976).

Darvel Series

The site is located at Lennoxtown, Scotland. Grid reference No. is NS 635773. The soil is cultivated as a garden. It belongs to the Darvel Association which is formed from fluvioglacial sands and gravels derived from carboniferous igneous and sedimentary rocks. The soil comes under the Darvel Series which has been classed as a freely drained brown forest soil of low base status.

Dreghorn Series

The site is situated at Troon, Ayrshire, Scotland. Grid reference No. is NS 328329. The soil is cultivated as a garden. It belongs to the Dreghorn Association which is developed from raised beach deposits. The series is Dreghorn which has been classed as freely drained brown forest soil.

Two soils, Darvel and Dreghorn series, were chosen for the pot experiment due to their differing texture and organic matter levels.

5.2.2 Collection and Preparation of Soil Samples

Darvel and Dreghorn soil samples were taken in the fresh condition from the upper 0-15 cm depth of the soil profile and were brought to the laboratory in labelled plastic bags as soon as possible. The samples were spread on clean plastic sheets and mixed thoroughly to minimize the effects of local variations and partially air dried sufficiently to pass through a sieve with 4 mm opening. Each soil was sieved to remove the larger inert material which is considered to have little effect on the chemical and nutritional status of soil. The samples were stored at 2°C until required.

Some selected properties of soils used in the experiment are shown in Tables 5.2 and 5.3.

Table 5.2 Properties of soils.

Soils	pH in water	LOI%	Moisture (%)
Darvel	6.4	8.82	29.82
Dreghorn	6.5	5.64	17.80

Table 5.3 Textural properties of soils (Mazumder,1992).

Soils	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Textural class
Darvel	33.5	0.0	22.0	24.5	sandy clay loam
Dreghorn	86.0	1.9	10.9	1.20	sand

Coarse sand > 0.18 mm, Fine sand = 0.18-0.05 mm, Silt = 0.05-0.002 mm and Clay < 0.002 mm (Khan, 1987).

5.2.3 Extraction of Macronutrients from Soils

The Soil Science Society of America (1979) has defined exchangeable ammonium as that ammonium which is extractable by neutral potassium salt solution for example, 0.5M potassium sulphate or 2M potassium chloride at room temperature. Flowers and Arnold (1983) also used 0.5M potassium sulphate during inorganic nitrogen analysis and they did not mention any significant interference of the sulphate ion. Therefore, this method was followed because it makes possible to determine ammonium, nitrate and nitrite in a single extract by applying colorimetric procedures of analysis.

Ammonium salts and weak acids such as acetic acid, ammonium acetate and ammonium nitrate are used for the extraction of available phosphate and potassium. Acetic acid 2.5% is the most popular extractant used by the Advisory Services in Scotland. Acetic acid is also the favourite extractant for phosphate and potassium extraction from soil in this department due to its merits

as described in the section 2.7. Therefore, 0.5M acetic acid was used as an extractant in this study.

5.2.3.1 Extraction of inorganic nitrogen

0.5M potassium sulphate was used in the extraction of ammonium, nitrate and nitrite from both soils. The extraction procedure can be seen in section 2.6.

5.2.3.2 Extraction of phosphorus and potassium

0.5M acetic acid solution was used for the extraction of phosphorus and potassium. The extraction procedure can be seen in section 2.7.

5.2.4. Determination of Macronutrients in Soil Extracts

Available nutrients were determined from Darvel and Dreghorn soils. Ammonium, nitrate, nitrite and phosphate were determined from soil extracts using the Technicon AutoAnalyzer II. Analytical procedure can be seen in sections 2.5.1., 2.5.2. and 2.5.3. Potassium was determined using flame photometer (see section, 2.5.4). Analytical conditions are given in Table 5.4.

Table 5.4 Analysis conditions for measuring nutrients in soil extracts.

Nutrients	Standard ($mg\ l^{-1}$)	Dilution factor	Extractant	Samples per hour
Ammonium	0 to 5	none	0.5M K_2SO_4	40
Nitrite	0 and 1	none	0.5M K_2SO_4	40
Nitrate	0 to 5	none	0.5M K_2SO_4	40
Phosphate	0 and 5	25*	0.5M Acetic acid	40
Potassium	0 to 25	none	0.5M acetic acid	-

* only in Dreghorn soil.

5.2.5 Pot Culture Techniques

350 and 450 g (on oven dry basis) of Darvel and Dreghorn soils, respectively were weighed into 10.0 cm internal diameter plastic pots. The pots were labelled as under:-

Darvel soil 1 to 20.

Dreghorn soil 21 to 40.

0.5 g perennial ryegrass seed (Lolium perenne L.) was sown in each pot on 6.7.91. The pots were covered by black polythene sheet to enhance germination. The germination started on 10.7.91. Pots were transferred to the greenhouse after germination completion. The walls of greenhouse were whitewashed by spraying white paint used by gardeners. The experimental detail is given below:-

Treatments = 4.

Treatment 1 = control.

Treatment 2 = 100:50:100 kg/ha N:P :K.

Treatment 3 = algal pond effluent.

Treatment 4 = synthetic effluent equivalent to algal pond effluent.

Replications = 4.

Experimental design = Randomized block.

LAYOUT PLAN

Block 1	21	26	6	16	31	1	11	36
Block 2	37	2	32	7	12	17	27	22
Block 3	33	28	38	8	3	18	23	13
Block 4	9	19	4	24	34	14	39	29
Block 5	15	25	5	40	20	35	30	10

Preparation of treatments

Treatment 1 Control.

It was an untreated control. Deionized water was applied throughout the course of the experiment.

Treatment 2 Standard fertilizer.

3.7025 g ammonium sulphate, 3.5978 g sodium hydrogen phosphate and 1.4966 g potassium chloride (all dried at 110°C) were added to a 1 litre volumetric flask and the volume was made with deionized water. Application of this solution at the rate of 100 ml per pot is equivalent to a fertilizer application of 100:50:100 kg/ha of N:P:K, respectively.

Treatment 3 Algal pond effluent.

Algal pond effluent was brought from the High-Rate Algal Ponds found in the greenhouse at Scottish Agricultural College, Auchincruive, Ayr. It was stored in the cold room at 2°C. Before each application, its chemical composition was found and applied at the rate of 100 ml per pot.

Treatment 4 Synthetic effluent equivalent to algal pond effluent.

Ammonium sulphate, sodium nitrate, sodium hydrogen phosphate and potassium chloride were dried at 110°C in the oven and cooled in a desiccator. 4.7165g ammonium sulphate, 6.0677 g sodium nitrate, 4.5832 g sodium hydrogen phosphate and 1.9066 g potassium chloride were weighed and added into a series of 1 litre volumetric flasks. The volume was made to the mark in each flask. These solutions were used as stock solutions to prepare the synthetic effluent treatment.

Ammonium-N, nitrate-N, phosphate-P and potassium equivalent to the amounts found in the algal pond effluent treatment were taken into a 1 litre volumetric flask from the stock solution (1000 mg l^{-1}) of each nutrient as described above and the volume was made to the mark with deionized water. Addition of 1.0 ml of the stock solution is equivalent to 1 mg l^{-1} in the fertilizer solution.

Application of treatments

A 100 ml bulb pipette was used in applying fertilizer treatments while a top pan balance was used for the algal pond treatment. All the applications were made to the top of the soil.

Control pots 1 to 5 and 21 to 25 were watered with deionized water at the time of fertilizer application and throughout the experiment.

The standard fertilizer at the rate of 100:50:100 kg per hectare of N:P:K in solution form was applied at the rate of 100 ml to each pot from pot No. 6 to 10 and 26 to 30 in a single application.

100 ml of algal pond effluent was applied to pots 11 to 15 and 31 to 35.

The synthetic effluent at the rate of 100 ml per pot was applied to pots from 16 to 20 and 36 to 40.

The total nutrients applied through algal pond effluent and the synthetic effluent up to cut 1 and cut 2 are shown in Table 5.5.

Table 5.5 Nutrients applied up to cut 1 and cut 2 of ryegrass.

Application Date	Nutrients			
	Ammonium (mg/pot)	Nitrate (mg/pot)	Phosphate (mg/pot)	Potassium (mg/pot)
Cut 1				
6.7.91	2.64	4.26	1.53	5.93
31.7.91	0.27	7.53	1.60	6.20
20.8.91	0.68	8.85	1.11	5.62
27.8.91	0.68	9.23	1.11	5.62
Total	4.27	29.87	5.35	23.37
Cut 2				
12.9.91	0.17	0.95	0.61	2.67
20.9.91	0.20	0.95	0.61	2.67
28.9.91	0.27	0.13	0.75	3.05
05.10.91	0.03	0.75	0.58	1.90
12.10.91	0.03	0.75	0.58	1.90
19.10.91	0.10	0.75	0.60	1.90
Total	0.80	4.28	3.73	14.09
Combined total	5.07	34.15	9.08	37.46

Watering system

The watering system was designed as shown in the Figure 5.1. Two gutter pipes were set on the greenhouse staging. The water was coming out from flasks held in a position that their mouth was touching the water surface in the gutter pipes so providing a constant water level. These flasks were held firmly with stands fixed near the end of the gutter pipes.

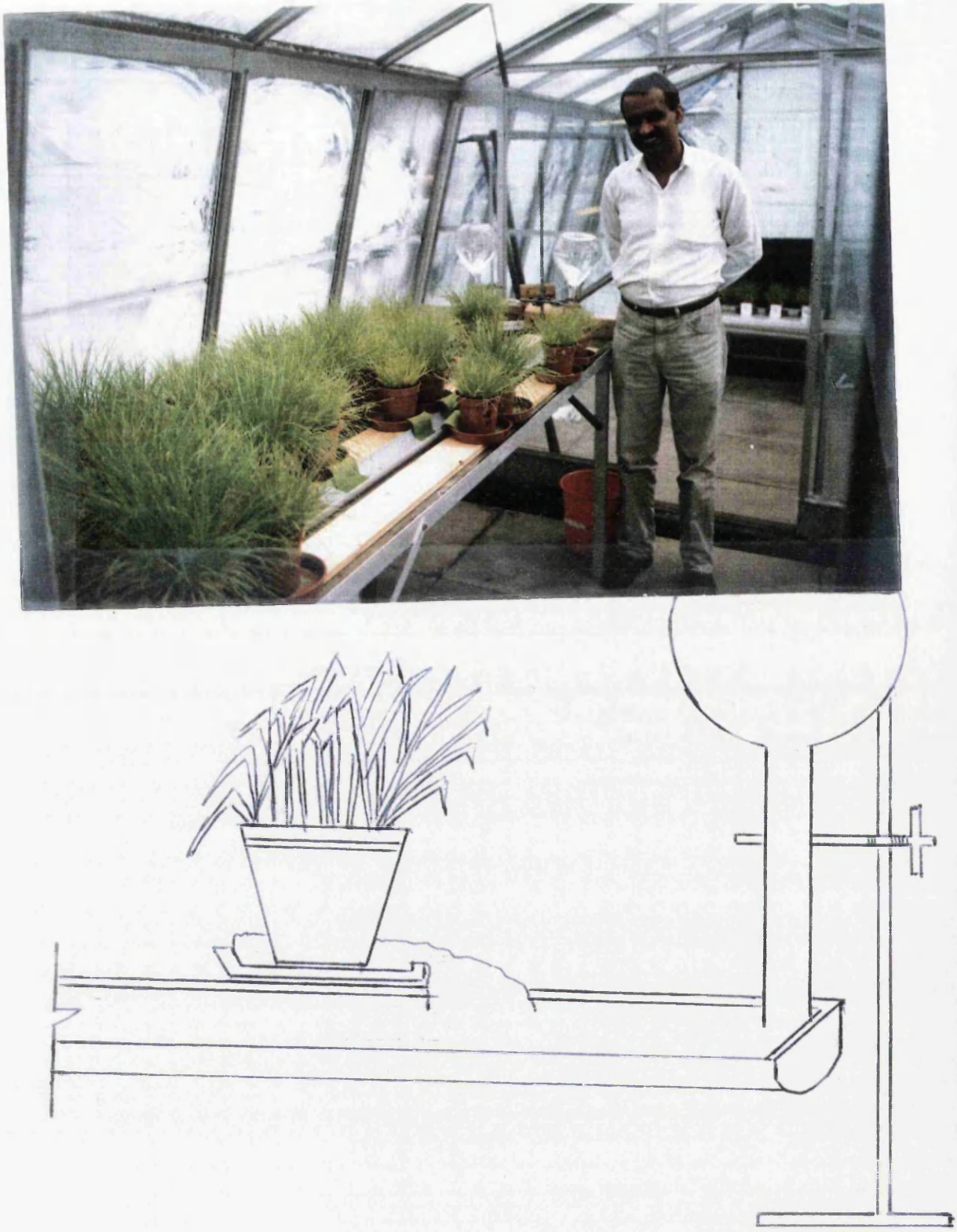

Figure 5.1 Watering system for pot experiment

The saucers with pots were elevated above the water level. An individual strip of capillary matting connected each saucer having a pot in it and water in the gutter pipe. The level of saucers was so adjusted that there was a water film in the capillary matting but the saucers were not flooded. As the water level of gutter pipe decreased by water uptake by plants, water flowed out the flasks. So, the water level in the gutter pipe and the water supply to the saucers remained constant. This watering system worked successfully throughout the course of the experiment. The water supply through capillary matting and the soil condition in each pot was checked twice a day. This system kept the soil moist but not waterlogged. Deionized water was applied throughout. As this study was carried out during summer, extra lighting was not arranged.

Harvesting

Fresh grass yield at cut 1 was recorded by cutting the grass with stainless steel scissors at 3.0 cm above the soil. The grass tops were wrapped in a 40 cm filter papers, weighed and then dried at 80°C for 48 hours. The dry matter yield was recorded by weighing. After cut 2, the contents of each pot were air dried. Then the roots were picked out by hand and by sieving the soil. Roots were thoroughly washed with deionized water to remove soil particles. The roots and grass tops of cut 2 were wrapped in 40 cm filter papers and dried at 80°C for 48 hours. Before analysis, the roots and tops were ground by using

an electric grinder. Ground samples were sealed in plastic bags and stored until required. The air dried soil of each pot was also sealed in plastic bags and stored until analysis.

5.2.6 Extraction of Macronutrients from Soils

Soil samples obtained from each pot were extracted for their measurement of ammonium, nitrate and nitrite, phosphate and potassium contents. The methods of extraction have been described in sections 2.6 and 2.7.

5.2.7 Acid Digestion of Herbage Samples

The grass and root samples were digested by the method of Bremner and Mulvaney (1982). This method includes oxidised forms of nitrogen such as nitrate. However, this method was modified using sodium sulphate instead of potassium sulphate in the catalyst mixture with an aim of measuring nitrogen, phosphate and potassium in a single acid digest of sample. The procedure has been described in section 2.8.

5.2.8 Determination of Macronutrients in Digests

Total ammonium, phosphate and potassium were determined from the digests obtained by the modified acid digestion procedure described in section 2.8.

5.2.8.1 Determination of total nitrogen in digests

Total nitrogen as ammonium was determined using the method described in section 2.5.1.

5.2.8.2 Determination of total phosphorus in digests

Total phosphorus was measured using the method in section 2.5.3.

5.2.8.3 Determination of total potassium in digests

Total potassium was determined by using flame photometer as described in section 2.5.4.

The conditions for analysis of acid digests are given in Table 5.6.

Table 5.6 Analysis conditions for measuring nutrients in acid digests.

Nutrients	Standards mg l^{-1}	Dilution factor	Samples per hour
Ammonium	0 to 100	20	40
Phosphate	0 to 50	20	40
Potassium	0 to 100	none	--

5.3 RESULTS AND DISCUSSION

The experiment was conducted to evaluate the algal pond effluent for supplying nutrients such as nitrogen, phosphorus and potassium to ryegrass under controlled conditions in the greenhouse. The results obtained are shown in Tables 5.7 to 5.16.

The fresh yield data shown in Tables 5.7 and 5.8 reveal that the standard fertilizer application was superior in producing green fodder yield to all other treatments in both soils. The algal pond effluent also had a positive effect on the growth of grass. It produced significantly more fresh yield compared with the control and even the synthetic effluent.

The individual data regarding the dry matter yield of grass, percent nitrogen, percent phosphorus and percent potassium in the grass harvested in cut 1, cut 2 and the roots are also shown in these tables. From these tables, total dry matter yield of grass was calculated by adding together the individual dry matter yield of grass at each cut. The yields of nitrogen, phosphorus and potassium shown as percent in each cut was first converted into mg/pot and then added together to obtain the total nutrients yields. The data so obtained are shown in Tables 5.9 and 5.10 for Darvel and Dreghorn soils, respectively.

The results shown in Table 5.9 reveal that the lowest dry matter yield was obtained in the control treatment while the highest yield was produced when the standard fertilizer was applied to the grass. The algal pond effluent and the synthetic effluent treatments,

although producing significantly higher yields than the control treatment, were not as effective as the standard fertilizer treatment. Pond effluent showed a small but statistically non significant increase in yield compared with the synthetic effluent treatment. The amounts of nitrogen and phosphorus in the grass followed the pattern of dry matter yield but that was not so for potassium. There was no significant difference in the amount of potassium harvested in algal pond effluent and chemical equivalent treatments.

The yields of dry matter and nutrients harvested from the Dreghorn soil shown in Table 5.10 were comparatively lower than those obtained for the Darvel soil. However, the yield trend among the treatments was similar. The standard fertilizer produced significantly the greatest yield and the control treatment the lowest yield. The algal pond effluent treatment did not differ significantly from the synthetic effluent treatment regarding the yields of dry matter, phosphorus and potassium. But the nitrogen yield in the grass as a result of applying the algal pond effluent was significantly more than nitrogen harvested in the grass in the synthetic effluent treatment.

It is obvious from these results that the highest yield of dry matter and nutrients was obtained in the standard fertilizer treatment in both soils. Neither the algal pond effluent nor the synthetic effluent treatment compete with the standard fertilizer treatment regarding the grass yield and its nutrients content. The greater

production of dry matter in pots which received the standard fertilizer application was certainly due to the presence of sufficient nutrients in the soil and their availability to plants. The significant increase in the yield of the algal pond effluent treatment over control was due to its available plant nutrients. On the other hand , the low yield in the effluent application and the synthetic effluent treatments compared to that obtained in the standard fertilizer treatment might be the low level of nutrients applied or their split application (Table 5.5). The total amounts of nitrogen, phosphorus and potassium applied through the algal pond effluent was 50%, 23% and 48%, respectively of the standard fertilizer application. As a result of the low nutrient level in the algal pond effluent and the synthetic effluent and their split application, yields were 48% and 44%, respectively of the yield obtained in the standard fertilizer in Darvel soil. Similarly, the algal pond effluent and the synthetic effluent application to the grass grown in Dreghorn soil produced 40% and 39%, respectively of that yield recorded in the standard fertilizer treatment. The low nutrients application compared to the standard fertilizer treatment was because of changes in the concentration of nutrients in the effluent, due to changes in the management of ponds made by the staff at the Scottish Agricultural College, Auchincruive, Ayr. These changes resulted in a very dilute effluent during the latter half of the experiment. The chemical analysis shown in the Table 5.5 reveals that the effluent applied after cut 1 was dirty water having very

low amounts of nutrients. Hence, the target of applying N:P:K at the rate of 100:50:100 kg/ha through algal pond effluent was not achieved.

Another important reason for the low yield might be the efficiency of nutrient use by the grass for the nutrients applied at intervals during the growth period of the crop. From the nitrogen, phosphorus and potassium data shown in Table 5.7 and 5.8, it is obvious that nutrients content of grass produced by the standard fertilizer was significantly lower than the concentration in the grass produced with algal pond effluent and the synthetic effluent treatments. This shows that application of nutrients at sowing as a single dressing had good effect on producing the dry matter yield. The application of algal pond effluent and the synthetic effluent at intervals to the grass was not as effective in producing yield as it increased the concentration of nitrogen, phosphorus and potassium in the herbage. It is clear from these results that there was a higher concentration of nutrients in the grass at cut 2 in both soils. As there was no second application of the standard fertilizer, increase in the nitrogen, phosphorus and potassium contents in the grass of standard fertilizer treatment must be the residual nutrients in both soils.

It is not clear if the effect of algal pond effluent is solely due to its available nitrogen, phosphorus and potassium or if there is a release of organic nutrients, as algal pond effluent proved significantly better than the synthetic effluent treatment in the Darvel but not in

the Dreghorn soil. However, these results show that the algal pond effluent is a useful material and may be applied to grass as organic irrigation alongwith chemical fertilizer. It is difficult to use it as an alternative material for the standard fertilizer.

Table 5.7 Grass and root yield and their nitrogen, phosphorus and potassium content in Darvel soil.

Treatments	Fresh yield (g/pot)	Dry matter yield (g/pot)	N (%)	P (%)	K (%)
Cut 1					
Control	4.26a	1.59a	0.92a	0.17a	1.4a
Standard fertilizer	13.93b	4.83b	0.85a	0.13b	1.0b
Algal pond effluent	11.16c	2.91c	1.26b	0.21c	1.6c
Synthetic effluent	9.89d	2.72c	1.17c	0.19c	1.7c
Cut 2					
Control	2.10a	0.50a	1.79a	0.71a	2.0a
Standard Fertilizer	17.20b	3.17b	1.90a	0.58b	2.3b
Algal pond effluent	5.32c	0.89c	2.38b	0.59b	1.6c
Synthetic effluent	3.99d	0.78c	2.08c	0.58b	2.3b
Roots					
Control	-	1.76a	0.65a	0.15a	0.3a
Standard fertilizer	-	7.40b	0.61a	0.14a	0.3a
Algal pond effluent	-	2.23a	0.81b	0.17a	0.2a
Synthetic effluent	-	2.26a	0.70c	0.15a	0.3a

Mean values followed by same letter within a column are not significantly different at 5% level using Fisher's LSD test.

Table 5.8 Grass and root yield and their nitrogen, phosphorus and potassium content in Dreghorn soil.

Treatments	Fresh yield (g/pot)	Dry matter yield (g/pot)	N (%)	P (%)	K (%)
Cut 1					
Control	3.02a	0.97a	0.81a	0.20a	1.4a
Standard fertilizer	12.70b	4.24b	0.78a	0.20a	0.9b
Algal pond effluent	8.68c	2.10c	1.29b	0.38b	1.3a
Synthetic effluent	7.14d	1.99c	1.18b	0.35b	1.5ac
Cut 2					
Control	1.75a	0.42a	1.47a	0.45a	1.8a
Standard fertilizer	17.64b	3.34b	1.68b	0.70c	1.9b
Algal pond effluent	5.67c	0.94c	2.10c	0.52a	1.3c
Synthetic effluent	4.20d	0.77d	1.85d	0.54a	1.8a
Roots					
Control	-	1.94a	0.68a	0.30a	0.3a
Standard fertilizer	-	12.87b	0.69a	0.30a	0.2b
Algal pond effluent	-	2.80c	0.73a	0.24b	0.2b
Synthetic effluent	-	2.91c	0.76a	0.28ab	0.3a

Mean values following by same letter within a column are not significantly different at 5% level using Fisher's LSD test.

Table 5.9 Total dry matter, nitrogen, phosphorus and potassium yield in grass in Darvel soil.

Treatments	Dry matter yield (g/pot)	N (mg/pot)	P (mg/pot)	K (mg/pot)
Control	2.10a	23.7a	6.3a	33a
Standard fertilizer	8.01b	102.0b	24.6b	124b
Algal pond effluent	3.81c	58.1c	11.3c	62c
Synthetic effluent	3.50d	48.1d	9.7d	63c

Mean values followed by same letter within a column are not significantly different at 5% level using Fisher's LSD test.

Table 5.10 Total dry matter, nitrogen, phosphorus and potassium yield in grass in Dreghorn soil.

Treatments	Dry matter yield (g/pot)	N (mg/pot)	P (mg/pot)	K (mg/pot)
Control	1.39a	14.0a	3.9a	21a
Standard fertilizer	7.59b	89.3b	31.7b	101b
Algal pond effluent	3.05c	46.7c	13.0c	40c
Synthetic effluent	2.75c	37.4d	11.2c	43c

Mean values followed by same letter within a column are not significantly different at 5% level using Fisher's LSD test.

The net release or loss of plant available nutrients in each soil was calculated on the basis of the initial plant available nutrients in the soil, nutrients added as the standard fertilizer, the algal pond effluent or the synthetic effluent, the nutrients recovered in the herbage and the plant available nutrients finally present in the soil.

The initial and final levels of the plant available nutrients were determined by extraction methods. Ammonium-nitrogen, nitrate-nitrogen and nitrite-nitrogen in soils before and after the pot experiment were determined by extraction with 0.5M potassium sulphate. The plant available phosphorus and potassium were extracted from soils with 0.5M acetic acid. These results are shown in Tables 5.11 and 5.12 for the Darvel and the Dreghorn soil, respectively. The amounts of nutrient applied as the standard fertilizer were 78.5, 39.25 and 78.5 mg/pot of N, P and K which were equivalent to 100:50:100 kg/ha of N:P:K. The amounts of nutrient applied as the algal pond effluent and the synthetic effluents are shown in Table 5.5. The nutrients recovered in the grass plus the roots are shown in Table 5.13 for the Darvel soil and Table 5.14 shows the nutrients recovered for the Dreghorn soil. The net release/immobilization or loss of the nutrients was calculated as follows:-

Net released/immobilized/lost = $G_n + R_n + FS_n - IS_n - A_n$

Where G_n = grass nutrients

R_n = roots nutrients

FS_n = final soil nutrients

IS_n = initial soil nutrients

A_n = added nutrients

The results so obtained for the Darvel soil are shown in Table 5.15 and for the Dreghorn soil in Table 5.16. The positive figures in the tables mean net mineralisation of nutrients from the soil whereas the negative values indicate net immobilization of nutrients by the soil or loss of nutrients from the soil.

The data in table 5.15 and 5.16 reveal that there was generally release of plant available nutrients from non available forms in the soil during the growth of the ryegrass. For example, the control treatment soil indicates net release of nitrogen, phosphorus and potassium into plant available forms and their supply to the growing grass. The control treatment in the Dreghorn soil showed a loss of phosphate. This loss could be due to the transformation of these ions into non available forms in this soil. It seems that phosphatic fertilizer might have been applied in the soil before its sampling for this experiment. The standard fertilizer application showed a stimulative effect on the release of the nutrients. The reason for the high net release of the nutrients and their uptake by the grass might be the elevated level of plant available nutrients supplied through the standard fertilizer. The other possibility for this release would be the development of the soil-plant relationship which further stimulated the breakdown of soil organic

constituents resulting in increased plant available nutrients and their uptake by the grass.

In making comparison between the effluents, it is clear that the algal pond effluent was superior to the synthetic effluent in releasing soil nitrogen and phosphorous. Their behaviour was similar for potassium release from the both soils. The effect of the application of both effluents on the release of nitrogen and phosphorus was variable compared with the control and the standard fertilizer. Potassium release from the soils due to the application of the effluents was lower than both the control and the standard fertilizer treatments. The variability in release/immobilization of nutrients in the effluent application compared with the control and the standard fertilizer might be due to the low nutrient and high water content of the effluents. The surplus water in both the effluent treatments might had a suppressing effect on the nutrient releasing behaviour of the two soils. Moreover, the excess water might have encouraged nitrogen loss by denitrification although the capillary matting watering system was designed to avoid nitrogen losses. The difference in net release/loss of phosphorus between the two soils might be due to the difference of the initial available phosphorus of these soils.

The greater amounts of nitrogen and phosphorus released from both soils by the application of the algal pond effluent compared with the synthetic effluent might be due to the decomposition of its organic matter content. Regarding the dry matter yield, the mineralisation of

nutrients from the algal pond effluent was not clear. The nutrient balance shows this effect more clearly, as there was greater net release of nitrogen and phosphorus from its organic matter content and their supply to the grass than for the synthetic effluent.

As the nitrogen concentration in the soil has a direct relationship to nitrogen content in the nearby waters, the nitrogen balance in the soils used in this study is an important consideration. It is clear from the final level of nutrients in these soils (Table 5.13 and 5.14) that available nitrogen decreased from its initial level in both soils in all treatments. Although this nitrogen present finally was in the nitrate form which is leachable under the field conditions, there was little difference between the treatments in both soils.

These results lead to the conclusion that there was good effect of the application of the algal pond effluent on the grass yield. It can not compete with the standard fertilizer application to the ryegrass yield and mineralisation of the plant available nutrients from the both soils. It can partially replace it if applied as organic irrigation to the ryegrass. There was a net release of nitrogen and phosphorus in the Darvel and the Dreghorn soils. This release was not greater than for the standard NPK fertilizer, therefore, it has less pollution hazard.

The volume to be applied to agricultural land can be controlled but the farmers will not know about its chemical composition. This will create difficulties in the

application of these results to field conditions because the effluent studied was of a synthetic nature. Treated Agricultural wastes as algal pond effluent can be applied to ryegrass as organic irrigation but there is uncertainty about its nutrients concentration. The cost of its application also is another factor to be considered alongwith its pollution potential particularly from its nitrogen content.

Table 5.11 Initial and final nitrogen, phosphorus and potassium in Darvel soil.

Treatments	N (mg/pot)	P (mg/pot)	K (mg/pot)
Initial	29.6	4.9	29
Final			
Control	3.5	3.7	13
Standard fertilizer	2.9	11.0	15
Algal pond effluent	4.3	3.5	15
Synthetic effluent	3.1	3.4	11

Table 5.12 Initial and final nitrogen, phosphorus and potassium in Dreghorn soil.

	N (mg/pot)	P (mg/pot)	K (mg/pot)
Initial	10.4	293	31
Final			
Control	5.0	268	19
Standard fertilizer	3.5	324	31
Algal pond effluent	4.6	286	25
Synthetic effluent	3.8	280	17

Table 5.13 Total dry matter, nitrogen, phosphorus and potassium yield in herbage plus roots in Darvel soil.

Treatments	Dry matter yield (g/pot)	N (mg/pot)	P (mg/pot)	K (mg/pot)
Control	3.86a	35.2a	8.9a	39.0a
Standard fertilizer	15.42b	147.0b	35.1b	144.0b
Algal pond effluent	6.04c	76.2c	15.0c	67.0c
Synthetic effluent	5.76c	64.1d	13.1c	69.0c

Mean values followed by same letter within a column are not significantly different at 5% level using Fisher's LSD test.

Table 5.14 Total dry matter, nitrogen, phosphorus and potassium yield in herbage plus roots in Dreghorn soil.

Treatments	Dry matter yield (g/pot)	N (mg/pot)	P (mg/pot)	K (mg/pot)
Control	3.33a	27.1a	9.7a	26.0a
Standard fertilizer	20.50b	178.0b	69.9b	131.0b
Algal pond effluent	5.85c	67.1c	19.6c	46.0c
Synthetic effluent	5.66c	59.6c	19.4c	51.0c

Mean values followed by same letter within a column are not significantly different at 5% level using Fisher's LSD test.

Table 5.15 Mineralized nitrogen, phosphorus and potassium in Darvel soil.

Treatments	N (mg/kg)	P (mg/kg)	K (mg/kg)
Control	27.1a	22.1a	64.0a
Standard fertilizer	120.0b	5.2b	148.0b
Algal pond effluent	34.4c	13.1c	44.0c
Synthetic effluent	-3.8d	7.5d	40.0c

Mean values followed by same letter within a column are not significantly different at 5% level using Fisher's LSD test.

Table 5.16 Mineralized nitrogen, phosphorus and potassium in Darvel soil.

Treatments	N (mg/kg)	P (mg/kg)	K (mg/kg)
Control	48.1a	-35.1a	32.0a
Standard fertilizer	206.0b	136.0b	117.0b
Algal pond effluent	49.0a	6.2c	5.6c
Synthetic effluent	30.6c	-7.1c	-1.0c

Mean values followed by same letter within a column are not significantly different at 5% level using Fisher's LSD test.

CHAPTER SIX

PLANT NUTRIENTS IN FARMYARD MANURE HEAPS FOUND IN PAKISTAN

6.1 INTRODUCTION

Farmyard manure is not properly stored and applied to agricultural crops in Pakistan. It is occasionally applied to crops such as chewing sugar-cane, vegetables and ornamental plants in the suburbs of big cities. Some farmers apply it to their fodder crops such as Egyptian clover and alfalfa on irrigated land in the rural areas of the country but there is no concept of applying it to crops grown in rainfed areas. Pakistan being a developing country, there is shortage of fuel, therefore, much of the farmyard manure produced is dried and burnt as fuel in homes.

Dera Ismail Khan is one of backward areas of the country. It has vast plain areas. The government has started developmental projects in this area, for example the Chasma right bank canal will be irrigating 100,000 hectares of land in 1994. Dera Ismail Khan is an arid region with hot summers typically up to temperatures of 35°C to 40°C and cold winters with mean temperatures typically of 10°C. The soils are mostly clayey with very low organic matter. So farmyard manure could be a source of organic matter. The crops raised in this district are shown in the Table 6.1.

Table 6.1 Crops grown in Dera Ismail Khan (Pakistan).

Canal/Tube-well irrigated area	Rainfed/Flooded Area
Sugar-cane	Wheat and barley
Cotton	Chickpea
Wheat and barley	Rape and mustard
Rice	Sorghum
Chickpea	Guar
Egyptian clover	
Maize	
Guar	
Citrus, mango and guava orchards.	
Vegetables	

The yield of crops grown in canal/tube-well irrigated areas are greater than those obtained in the rainfed/flooded areas. This is mostly because of use of improved varieties, use of advanced technology, use of fertilizer, manure application to some crops, no water deficiency and use of plant protection measures. Although the crops yields in the irrigated areas are more than the non irrigated areas, they are low compared with the others areas of Pakistan such as the Punjab. The reasons for the low yield are many such as the unavailability of fertilizers, the high price of fertilizer due to black marketing, lack of suitable information about the benefits

of the fertilizer etc. The prices of different fertilizers used in Dera Ismail Khan are given in Table 6.2.

Table 6.2 Price of 50 kg fertilizer bag (1992-93).

Fertilizer	Price(£)
Single super phosphate	2.0
Ammonium sulphate	1.0
Urea	4.5
Sulphate of potash	3.5
Diammonium phosphate	6.0

Use of manure in irrigated areas is due to the availability of fuel wood from trees planted along the land borders and water channels. These trees are trimmed annually to get fuel for the winter season. Whereas in unirrigated areas, the wood is sold to the brick makers to make some money and to buy domestic things and food items such as wheat flour or grains. The farmers here meet their fuel demand by burning bricks made from animal dung. Those who have more animals with more manure production make money by selling manure bricks to others. Most of the housewives consider it as their pocket money.

In Britain, the farmers are well informed about waste disposal, crops yields, soil fertility status etc. by the extension and the research departments. They can get any information they need. The farmers are educated and

know about the merits and demerits of the farm wastes. They are well aware about the storage methods and handling of the farmyard manure or slurry. There is legislation to prevent pollution by farm wastes. The farmers make modifications in their farm activities according to the scientific advice available to them.

On the other hand, Pakistani farmers are lacking this type of information. Farmyard manure is not properly stored and handled. The solid portion of the animal farmyard manure is dried and burnt as fuel. The rest of it is heaped in the open air and not covered. They do not know the nutrient level of these heaps. In many places, instead of applying to crops, it is used for filling the pits of the houseyard and covered with a soil layer. Therefore, farmers of this area do need information about benefits of the farmyard manure. This information must be obtained by carrying out the research about the following points:-

Whether present methods of storage of farmyard manure are good enough for the conservation of the valuable plant nutrients or not?

If not, how it should be stored?

Is its application to crops is beneficial or not?

Is the application of the farmyard manure alongwith chemical fertilizer beneficial for crops or not?

Is it necessary to burn the farmyard manure if an alternative fuel is not available?

If burnt should ash be collected or not?

Which is the more economical either farmyard manure burning or buying fuel wood?

The farmers of Dera Ismail Khan need answers to the above questions. After having the required information and the availability of canal water as mentioned earlier, they would agree to use the farmyard manure as plant nutrients source. So far, no research on farmyard manure for its plant nutrient levels has been conducted by either the Faculty of Agriculture, Gomal University or the Research and Extension Departments of the province (North West Frontier Province) Pakistan.

Keeping in view the possible future importance of farmyard manure in Dera Ismail Khan, it seemed worthwhile to carry out research experiments to evaluate the farmyard manure for its major plant nutrients. These experiments are summarised below:-

Experiment 1 Survey of farmyard manure heaps

To determine the levels of nutrients in typical farmyard manure heaps and to assess how typical management practices influence nutrient levels.

Experiment 2 Nutrients content of farmyard manure

To determine how much nitrogen, phosphorus and potassium are present in the fresh dung and urine produced by housed livestock, how much is retained in the farmyard manure and how much is lost through drainage.

Experiment 3 Production of farmyard manure

To determine the production of the farmyard manure per animal per night and to investigate the nitrogen, phosphorus and potassium in it.

Experiment 4 Storage of farmyard manure.

To determine the losses of nitrogen, phosphorus and potassium from farmyard manure stored in different ways.

6.2 METHODS

6.2.1 Sampling from Farmyard Manure Heaps

The heap was divided into different portions for sampling. The number of samples taken from each portion were in proportion to the size of each part of the heap. The rubbish from the heap surface was removed. Upto five samples were taken from each portion of the heap depending on the relative size of each portion, so that the pooled sample reflected the relative sizes of the portion of the heap. The sampler was a hollow steel pipe (10 cm diameter) with slightly angled sharp edges which supported the trapped sample. This sampler was forced into five points of the same heap. All the samples taken from the same heap were mixed thoroughly and pooled in a large plastic bag and refrigerated in the Food Research laboratory until sub-sampling for analysis.

6.2.2 % Moisture Content

10 g samples of farmyard manure were weighed in cleaned and dried basins. These basins were transferred into the oven. The temperature of the oven was 105°C. The farmyard manure was dried overnight, cooled in a desiccator and reweighed by top pan balance to 0.01 g. The % moisture was calculated as follows:-

$$\% \text{ Moisture} = \frac{\text{fresh weight - oven dry weight}}{\text{fresh weight}} \times 100$$

6.2.3 pH Determination

5 g of each sample was weighed into clean 100 ml plastic bottles. 25 ml distilled water was added to each bottle with a graduated cylinder. The samples were shaken for 30 minutes on a vertical reciprocating shaker at room temperature of 25°C in the laboratory. The pH/mV meter (Jenway, Model 3010) was calibrated with buffers of pH 7.01 and 9.2. After calibration, the electrode was immersed directly into the sample suspension and the readings were recorded. The electrode was rinsed with distilled water between sample readings.

6.2.4 Procedure for Extraction of Available Nitrogen, Phosphorus and Potssium in Farmyard Manure and Urine

6.2.4.1 Reagents

0.025M sulphuric acid

1.4 ml concentrated sulphuric acid (AR) was dissolved in 1 litre of distilled water.

6.2.4.2 Procedure for extraction of farmyard manure

5 g farmyard manure samples were weighed by top pan balance into 100 ml plastic bottles. 100 ml of 0.025M sulphuric acid was added with a graduated cylinder to each sample bottle. The samples were shaken on a mechanical shaker for 30 minutes. Then samples were filtered through Whatman No.1 filter paper. These filters were washed with 0.5 M sulphuric acid before use in filtration (Shah,

1988). The filtrates were stored in the fridge until analysis.

6.2.4.3 Procedure for extraction of urine

95 ml of 0.025M sulphuric acid was added to 5 g urine samples. The rest of the procedure was the same as in the farmyard manure extraction described as above.

6.2.5 Procedure for Kjeldahl Digestion of Farmyard manure and Urine

The farmyard manure and urine samples were digested by the method of Bremner and Mulvaney (1982). This method includes oxidised forms of nitrogen such as nitrate. However, this method was slightly modified using a sodium sulphate-catalyst mixture instead of potassium sulphate mixture. This modification facilitated the measurement of nitrogen, phosphorus and potassium in the same digest.

6.2.5.1 Reagents

Salicylic acid-sulphuric acid mixture

25 g salicylic acid was dissolved in litre of concentrated sulphuric acid in a beaker. The mixture was poured carefully into a glass bottle.

Sodium thiosulphate pentahydrate

Sodium sulphate-copper sulphate mixture

100 g sodium sulphate was finely ground with 10.0 g of copper sulphate in a mortar and pestle. The mixture was stored in a glass bottle.

6.2.5.2 Procedure for digestion of air dried farmyard manure

0.2 g of air dried and finely ground samples were poured by a glass funnel with a long stem into Kjeldahl digestion flasks. 5 ml concentrated sulphuric plus salicylic acid mixture was added to each sample. This mixture was left overnight.

0.5 g sodium thiosulphate was added through a long neck glass funnel. This mixture was heated gently using Kjeldahl nitrogen digesting apparatus with electric heating until the frothing ceased. The flasks were removed from the heating apparatus and allowed to cool. 1.0 g sodium sulphate plus copper sulphate (20:1) catalyst mixture was added to each sample. This mixture was again heated until it cleared, then for a further 1 hour. The flasks were transferred to the cooling stand for 15 minutes and 25 ml of distilled water was added very carefully. Then the digests were transferred into 100 ml volumetric flasks and the volume was made up to the mark. The digests were filtered through Whatman No.1 filter papers which were already washed with 0.5M sulphuric acid.

6.2.5.3 Procedure for digestion of wet farmyard manure

Samples were mixed thoroughly. 2.5 g of each sample was poured into kjeldahl flasks. 25 ml concentrated sulphuric acid and 5 g catalyst mixture (100 g sodium sulphate plus 5 g copper sulphate) was added to each flask. The mixture was heated gently until frothing ceased and more strongly until it cleared. The heat was reduced, so that sulphuric acid was condensing about one third way up the stem of the flask. This heating continued for 1 hour.

The digests were cooled for 15 minutes and 50 ml of distilled water was added very carefully to each flask to dissolve the digest. These digests were transferred to 500 ml volumetric flasks and made up to volume. The digests were filtered through Whatman No.1 filter papers which were already washed with 0.5M sulphuric acid.

6.2.5.4 Procedure for digestion of urine

Samples were mixed thoroughly by stirring. 5 g samples was poured into Kjeldahl flasks. 5 ml concentrated sulphuric acid and 1 g catalyst mixture (100 g sodium sulphate plus 5 g copper sulphate) was added to each sample. Mixture was heated gently to evaporate the water then the same procedure as described in the digestion of fresh farmyard manure samples was followed.

6.2.6 Determination of Available and Total Nitrogen

6.2.6.1 Reagents

Nessler's reagent

55 g mercuric iodide and 41 g potassium iodide were dissolved in about 400 ml distilled water. 144 g sodium hydroxide was dissolved in 400 ml distilled water and was allowed to cool. The two solutions were mixed and stirred continuously in a 1 litre beaker and volume was made. Then this solution was stored in a dark glass bottle. The clear supernatant solution was used and care was made not to disturb the sediment during use.

Complexing reagent

25 g sodium potassium tartrate and 25 g tri-sodium citrate were dissolved in distilled water and volume was made in 100 ml volumetric flask.

0.05M sodium hydroxide

2.0 g sodium hydroxide was dissolved in distilled water and volume was made to 1 litre.

1.8M sodium hydroxide

18 g sodium hydroxide was dissolved in distilled water and volume was made in a 250 ml volumetric flask.

0.1M sodium hydroxide

4.0 g sodium hydroxide was dissolved in distilled water and volume was made in 1 litre volumetric flask.

50 % sodium hydroxide

500 g sodium hydroxide was dissolved in distilled water and volume was made in 1 litre volumetric flask.

Acid blank solution

1.0 g sodium sulphate was dissolved in about 80 ml distilled water. 5.0 ml concentrated sulphuric acid was added to this solution. When cooled, it was diluted to 100 ml in volumetric flask.

Stock ammonium-N solution (1000 mg l^{-1})

Ammonium sulphate was dried in an oven at 100°C for 1 hour and cooled in a desiccator. 4.717 g of the dried salt was dissolved in distilled water and volume was made to 1 litre in volumetric flask. This solution was stored in the fridge.

Standard ammonium-N solution (10μg cm^{-3})

1.0 ml of stock solution was diluted to 100 ml in volumetric flask with distilled water.

6.2.6.2 Procedure for determination of available ammonium-nitrogen in 0.025M sulphuric acid extracts by Nessler's method

Calibration curve (0 - 50 μg NH_4-N)

0, 1, 2, 3, 4 and 5 ml of 10 μg cm^{-3} NH_4-N standard solution was pipetted into a series of 50 ml volumetric flasks. 1.0 ml of extracting solution was added to each flask. 1.0 ml of complexing reagent was added to each flask. It was diluted to approximately 40 ml with distilled water and was mixed thoroughly. 1.0 ml of 0.05M sodium hydroxide was added to each solution. 1.0 ml of Nessler's reagent was added immediately after the addition of sodium hydroxide. The volume was made with distilled water upto the mark. These solutions were allowed to stand for 15 minutes for full colour development. The colour was measured at 425 nm using a WPA SK110 auto zeroing spectrophotometer. The plastic cuvettes used for filling the solution for measurement were of 10 mm path length.

Samples (0 - 50 μg N cm^{-3})

1.0 ml aliquots of each sample extract were pipetted into a series of 50 ml volumetric flasks. 1.0 ml of complexing reagent was added to each flask. It was diluted to approximately 40 ml with distilled water and was mixed thoroughly. 1.0 ml of 0.05M sodium hydroxide was added to each solution. 1.0 ml of Nessler's reagent was added immediately after the addition of sodium hydroxide. The volume was made with distilled water upto the mark. These

solutions were allowed to stand for 15 minutes for full colour development. The colour was measured at 425 nm using SK110 auto zeroing spectrophotometer.

6.2.6.3 Procedure for determination of total nitrogen in Kjeldahl digests by Nessler's method.

Calibration curve (0 - 50μg NH_4-N)

0, 1, 2, 3, 4 and 5 ml of 10μg cm^{-3} NH_4-N standard solution was pipetted into a series of 50 ml volumetric flasks. 1.0 ml of complexing reagent and 1.0 ml of acid blank solution was added to each flask. It was diluted to approximately 40 ml with distilled water and was mixed thoroughly. 1.0 ml of 1.8M sodium hydroxide was added to each solution. 1.0 ml of Nessler's reagent was added immediately after the addition of sodium hydroxide. Then volume was made up to the mark. These solutions were allowed to stand for 15 minutes for full colour development. The colour was measured at 425 nm using the SK110 auto zeroing spectrophotometer. The plastic cuvettes used for filling the solution for measurement were of 10 mm path length.

Samples (0 - 50μg N cm^{-3})

1.0 ml of aliquot of each sample digest was pipetted into a series of 50 ml volumetric flasks. 1.0 ml of complexing reagent was added to each flask. It was diluted to approximately 40 ml with distilled water and was mixed thoroughly. 1.0 ml of 1.8M sodium hydroxide was

added to each solution. 1.0 ml of Nessler's reagent was added immediately after the addition of sodium hydroxide. Then volume was made up to the mark. These solutions were allowed to stand for 15 minutes for full colour development. The colour was measured at 425 nm using the SK110 auto zeroing spectrophotometer.

6.2.7 Determination of Available and Total Nitrogen by Distillation

6.2.7.1 Reagents

Magnesium oxide (MgO)

MgO was pretreated by heating at 700°C for two hours, cooled in a desiccator and stored in an air tight bottle.

0.025M sulphuric acid

1.4 ml concentrated sulphuric acid was diluted to 1 litre in a volumetric flask with distilled water.

0.025M sulphuric acid

The 0.025M sulphuric acid was prepared by diluting 10 ml of 0.025M sulphuric acid in 100 ml of distilled water in a volumetric flask.

Boric acid solution (2%)

20.0 g boric acid was dissolved in distilled water and volume was made to 1 litre in a volumetric flask.

Boric acid indicator mixture solution

To 250 ml of 2% boric acid, 1.5 ml of 0.05% methylene blue in ethanol was added followed by 3 ml of 0.1% methyl red in ethanol and 1 ml of 0.1M NaOH solution. This solution should turn green when mixed with an equal volume of distilled water.

Stock ammonium-N solution (1000 mg l^{-1})

Ammonium sulphate was dried in an oven at 100°C for 1 hour and cooled in a desiccator. 4.717 g of the dried salt was dissolved in distilled water and volume was made to 1 litre in volumetric flask.

Standard ammonium-N solution (5 mg l^{-1})

5.0 ml of stock solution was diluted to 100 ml in volumetric flask with distilled water.

Tris hydroxymethyl amino methane (THAM).

This chemical was not available.

Standardization of sulphuric acid

Due to the unavailability of the THAM, the sulphuric acid used for titration could not be standardized.

6.2.7.2 Procedure for determination of ammonium-N in 0.025M sulphuric acid extracts

The distillation apparatus used for determination of nitrogen in extracts and digests has been shown in fig 6.1.

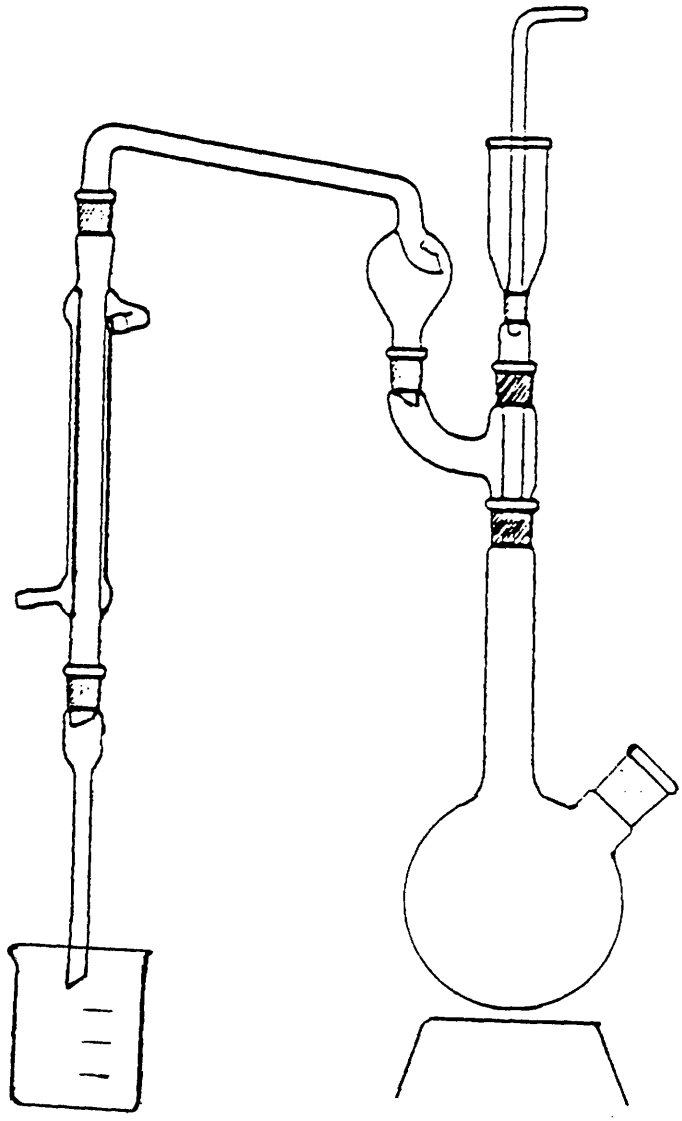

Figure 6.1 Distillation apparatus for nitrogen determination

5 ml of boric acid indicator mixture was taken into a 50 ml beaker and the beaker placed under the condenser of the distillation apparatus so that the end of the condenser was below the surface of the boric acid mixture.

10.0 ml of extract was taken by a 10 ml bulb pipette into a 250 ml distillation flask. 10 ml of 0.05M NaOH was added to the sample and followed by addition of 0.2 g MgO. The flask was attached to the distillation apparatus as shown in Figure 6.1. The distillation was continued and distillate was collected for 5 minutes. The ammonium-N was determined by titration with 0.025M sulphuric acid from a 50 ml burette graduated to 0.1 ml. The colour change at the endpoint was from green to a permanent, faint pink.

To perform a blank distillation, the same procedure was followed as described for the sample by distillation of 10 ml of extract blanks.

6.2.7.3 Procedure for determination of nitrate-N and nitrite-N in 0.025N sulphuric acid extracts

After removal of NH_4-N from the sample as described above , 0.2 g of Devarda alloy was added to the flask through side arm by a glass funnel with long stem in order to reduce nitrate and nitrite nitrogen into ammonium-N. 5 ml of boric acid was taken in a new beaker and placed under the condenser. The distillation was repeated for the determination of nitrate and nitrite, collecting the distillate for further 5 minutes. The ammonium-N so

collected was determined by titration with 0.0025M sulphuric acid.

6.2.7.4 Procedure for determination of total nitrogen in Kjeldahl digests

5 ml of boric acid indicator mixture was taken into a 50 ml beaker and the beaker placed under the condenser of the distillation apparatus so that the end of the condenser was below the surface of the boric acid mixture.

A 10.0 ml of aliquot of the digest was taken by a 10 ml bulb pipette into a distillation flask. 5 ml of 50% NaOH was added to the sample. The flask was attached to the distillation apparatus as shown in Figure 6.1. The distillation was continued and distillate was collected for 5 minutes. The ammonium-N was determined by titration with 0.0025M sulphuric acid from a 50 ml micro burette graduated in 0.1 ml divisions. The colour change at the endpoint was from green to a permanent, faint pink.

To analyse a blank, the same procedure was followed as described for the sample by taking 10 ml of blank digest solution.

Calculation

The results were calculated by the following formula

$$N\ (\mu g/g) = \frac{(\text{titre-blank titre}) \times 70^{*} \times \text{total extract volume}}{\text{Weight of sample} \times \text{volume of aliquot distiled}}$$

* a 70 μg N corresponds to 0.0025M sulphuric acid

6.2.8 Determination of Available and Total Phosphorus

Available phosphorous was measured in dilute sulphuric acid extracts and total P was determined in Kjeldahl digests.

6.2.8.1 Reagents

Mixed Reagent

151 ml concentrated sulphuric acid (AR) was added slowly to approximately 500 ml distilled water. It was cooled and 20 g ammonium molybdate dissolved in 200 ml distilled water was added. 0.4 g potassium antimony tartrate was dissolved in approximately 100 ml distilled water and added carefully to the acid molybdate solution, with mixing by a glass rod. The volume was made to 1 litre and stored in a brown bottle.

Complete Reagent

1.5 g ascorbic acid (AR) was dissolved in 100 ml of mixed reagent at each time of P determination in the samples.

Stock P solution (1000 mg l^{-1})

Potassium dihydrogen orthophosphate was dried in an oven at 100°C for 1 hour. 1.098 g dried and cooled salt was dissolved in approximately 200 ml distilled water and volume was made to 250 ml in a volumetric flask. This solution was stored in the fridge.

Working P standard solution (10 μg P cm^{-3})

1 ml of stock P solution was diluted to 100 ml in a volumetric flask.

6.2.8.2 Procedure for determination of available phosphorus in 0.025M sulphuric acid extracts

Calibration curve (0 - 50 μg P)

0, 1, 2, 3, 4 and 5 ml of 10 μg cm^{-3} P standard solution was pipetted into a series of 50 ml volumetric flasks. It was diluted to approximately 40 ml with distilled water and was mixed thoroughly. 5.0 ml of complete reagent was added to each standard solution and then the volume was made to the mark. These solutions were allowed to stand for 30 minutes for full colour development. The colour was measured at 880 nm using a WPA SK110 auto zeroing spectrophotometer. The plastic cuvettes used for filling the solution for measurement were of 10 mm path length.

Samples (0 - 50 μg N cm^{-3})

1.0 ml of aliquot of each sample extract was pipetted into a series of 50 ml volumetric flasks. It was diluted to approximately 40 ml with distilled water and was mixed thoroughly. 5.0 ml of complete reagent was added to each standard solution and then the volume was made to the mark. These solutions were allowed to stand for 30 minutes for full colour development. The colour was measured at 880 nm using the SK110 auto zeroing spectrophotometer.

6.2.8.3 Procedure for determination of total phosphorus in Kjeldahl digests

Calibration curve (0 - 50 μg P)

0, 1, 2, 3, 4 and 5 ml of 10 μg cm^{-3} P standard solution was pipetted into a series of 50 ml volumetric flasks. 1.0 ml of acid blank was added to each flask. It was diluted to approximately 40 ml with distilled water and was mixed thoroughly. 1.0 ml of 1.8M NaOH was added to each solution to neutralize the acid. 5.0 ml of complete reagent was added to each standard solution and then the volume was made to the mark. These solutions were allowed to stand for 30 minutes for full colour development. The colour was measured at 880 nm using SK110 auto zeroing spectrophotometer. The plastic cuvettes used for filling the solution for measurement were of 10 mm path length.

Samples (0 - 50 μg P cm^{-3})

1.0 ml of aliquot of each sample extract was pipetted into a series of 50 ml volumetric flasks. It was diluted to approximately 40 ml with distilled water and was mixed thoroughly. 1.0 ml of 1.8M NaOH was added to each solution to neutralize the acid. 5.0 ml of complete reagent was added to each standard solution and then the volume was made to the mark. These solutions were allowed to stand for 30 minutes for full colour development. The colour was measured at 880 nm using SK110 auto zeroing spectrophotometer.

6.2.9 Determination of Available and Total Potassium

6.2.9.1 Reagents

Stock K solution (1000 mg l^{-1})

Potassium dihydrogen orthophosphate was dried in an oven at 100°C for 1 hour and cooled in a desiccator. 1.740 g of the dried salt was dissolved in 200 ml distilled water and volume was made to 250 ml in a volumetric flask. This solution was stored in the fridge.

K standard curve (0-50 mg l^{-1})

0, 1, 2, 3, 4 and 5 ml of stock K solution was diluted in a series of 100 ml volumetric flasks with 0.025M sulphuric acid.

6.2.9.2 Procedure for determination of available potassium in 0.025M sulphuric acid extracts

The flame photometer PFP 7 (Jenway Ltd.) was calibrated with standard calibration solutions (0 - 50 mg l^{-1}). The blanks and extracts were aspirated and the readings were recorded.

6.2.9.3 Procedure for determination of total potassium in Kjeldahl digests

K standard curve (0 - 100 mg l^{-1})

0, 2, 4, 6, 8 and 10 ml of stock K solution was diluted in a series of 100 ml volumetric flasks with distilled water.

These solutions had equal concentration of reagents as in the digests.

The flame photometer PFP 7 (Jenway Ltd.) was calibrated with standard calibration solutions (0 - 100 mg l^{-1}). The blanks and digests were aspirated and the readings were recorded.

The results were calculated using the K calibration programme on BBC computer in this Department.

6.2.10 Description of Experiments

6.2.10.1 Experiment 1 (survey of farmyard manure heaps)

This experiment was conducted to investigate the levels of nitrogen, phosphorus and potassium in farmyard manure heaps. A small survey was made in the village of Lundah Sharif located 15 miles to the south of Dera Ismail Khan city. There is no canal or tube-well irrigated agriculture in this area. The crops are grown on the moisture conserved from rain or torrent floods. This survey was conducted by putting the following questions to the five farmers:-

How many animals are domesticated by the farmer ?

What kind of animals the farmer domesticate ?

Is bedding used during animal housing?

What kind of bedding he uses ?

What kind of feeding is given to the animals ?

How much portion of the farmyard manure is burnt?

How the farmyard manure is stored ?

What will be the future use of the farmyard manure ?

What would be the price of the farmyard manure heap?

The same questions were put to the Farm manager, Faculty of Agriculture, located in the city of Dera Ismail Khan. The farmyard manure produced by buffaloes domesticated by the Faculty was included in this survey as heap No. 6. Samples were taken from each heap of the farmyard manure. Available and total macronutrients in the samples of surveyed farmyard manure heaps were determined.

6.2.10.2 Experiment 2 (nutrient content of farmyard manure)

Experiment 2 was conducted with the aim of finding out the amounts of nitrogen, phosphorus and potassium in the fresh dung and urine produced by housed buffaloes. This experiment was conducted at the Faculty of Agriculture, Gomal University, Dera Ismail Khan. After selecting 4 animals (3 female and 1 male buffaloes), the animals were isolated from the other animals and were tied separately. The fresh dung and urine were sampled and analysed to investigate the total and available nitrogen, phosphorus and potassium levels. The detail of these animals is given in Table 6.3.

Table 6.3 Detail of the animals selected for experiment 2.

Animal No	Name	Birth date	Sex	Condition
1	Shama	9.10.83	Female	Pregnant Milking
2	Silver	23.5.89	Female	Pregnant
3	Golden	5.10.90	Female	-
4	Ranjha	27.12.83	Male	-

The animal feeding was not standardised. Wheat straw and green fodder was being fed to the animals during the course of the experiment. The detail of the fodder fed during the course of this experiment is as under:-

6.5.92 to 12.5 92	Egyptian clover
13.5.92 to 17.5.92	Forage maize
18.5.92 to 22.5.92	Egyptian clover
23.5.92 to 12.7.92	No fodder

The general detail of individual animal feeding is given in Table 6.4.

Table 6.4 Feeding of the 4 animals used in experiment 2

Animal Number	Feeding detail
1 (milking buffalo)	6 kg cottonseed cake plus wheat husk plus wheat straw plus Egyptian clover plus maize fodder.
2 (pregnant buffalo)	wheat straw plus Egyptian clover plus maize fodder.
3 (non milking buffalo)	wheat straw plus Egyptian clover plus maize fodder.
4 (Male buffalo)	wheat straw plus Egyptian clover plus maize fodder

Usually the buffaloes are tethered on the concrete based sleeping area during the night. However, when the animals are not free for grazing, they are also tethered during the day. If there is a crop in the field for grazing, these animals are allowed free in the crop for grazing for 3-4 hours. When there is no crop for grazing, the animals are made free for exercise for one to two hours daily. Then they are brought back to the housing area, allowed to drink and washed in the drinking area by the animal watchers. Again the animals are tethered on the concrete base as described above.

For the collection of dung and urine the animals were tethered separately on the concrete based sleeping area. They were watched until they dropped their dung. The dung was mixed thoroughly by hand and samples were taken and put into separate polythene bags for each animal. The available nutrients were determined immediately and samples were stored in the fridge for total nutrients determination.

Animals were watched until they excreted urine. Urine was collected in a clean large beaker and transferred into clean glass bottle for each animal when analysis of individual urine samples was required. For making a collective sample of all 4 animals, urine of each animal was mixed together in a big beaker. Available macronutrients were determined in the subsamples of urine and samples were stored in the fridge until acid digestion for determination of total nutrients.

6.2.10.3 Experiment 3 (production of farmyard manure)

The objective of this experiment was to find out the amount and nutrient composition of farmyard manure produced from dung and urine with and without bedding by a group of four buffaloes. The farmyard manure produced from four buffaloes during the course of experiment 2 was weighed daily by a spring balance and a plastic bucket. After taking the weight, the farmyard manure was mixed and heaped on the concrete floor. Samples were taken from 10 points of this fresh temporary heap with a soil corer. Samples were pooled in a polythene bag. Available macronutrients were determined by taking subsamples and the rest of the sample was stored in the fridge until acid digestion for total nutrients determination.

The concrete based sleeping area sloped towards the back of animals. A pit was dug at the end of drain running at the edge of the concrete floor where these four selected animals were tied during the night. The pit was also covered during the urine collection time. A big tin jar with a polythene bag inside it was fixed in this pit. The urine flowing from the floor surface to the drain was collected in this jar until morning.

In the first part of this experiment the farmyard manure was produced by four buffaloes without bedding. During the second part of this experiment sawdust was used as bedding to prevent the loss of urine.

6.2.10.4 Experiment 4 (storage of farmyard manure)

This was a summer storage experiment. It was conducted to examine the effect of storing the farmyard manure, produced without night bedding (the first part of the experiment 3) in different ways. The farmyard manure was collected between 6.5.92 and 7.6.92. The farmyard manure produced during the first part of experiment 3 was collected and heaped on the land surface in the area where it was to be stored. Before starting up experiment 4, the farmyard manure was mixed with the help of a spade. Then it was divided into 4 approximately equal portions. Each experimental heap was stored in a 90 cm deep ditch according to the following treatments.

Treatment 1 covered with soil.
Treatment 2 covered with wheat straw.
Treatment 3 covered with plastic sheet.
Treatment 4 uncovered.

Samples were taken from each stored farmyard manure heap by a soil corer (a metre long with 5 cm diameter) to determine the initial levels of nitrogen, phosphorus and potassium. Subsequent sampling was done using the same sampler and analysis was carried out to determine the possible changes occurring in the stored farmyard manure heaps.

6.3 RESULTS AND DISCUSSION

6.3.1 Experiment 1 (Survy of Farmyard Manure Heaps)

The survey of farmyard manure heaps conducted in the village of Lundah Sharif Dera Ismail Khan, Pakistan indicated that the farmers domesticate different kind of animals. They do not follow standardised animal rationing. The animals are housed from the end of October to March. Green fodder if available is fed to the animals, otherwise wheat straw is fed.

The farmyard manure produced by these animals is not stored properly and applied to the land. Much of it is dried as dung bricks and burnt as fuel for heating and cooking. The rest of it is stored as heaps either in the houseyard or on the corner of the street or in a deep point of nearby land in the open air and uncovered. The samples collected from these heaps were analysed for their properties and nutrient contents. The results of the survey of each heap are given as under:-

HEAP 1 OWNER MR. MITHU KHAN

Age of heap	4 years
Place of heap	New site out of doors
Number of animals	6
Kind of animals	2 Bullocks , 2 cows and 2 calves
Bedding used	Dried farmyard manure
Fodder	Egyptian clover
Future use of heap	To fill pits
Portion used as fuel	50%
Quality of heap	Not good
Sale price of heap	£ 6.00

HEAP 2 OWNER MR. GHULAM HUSSAIN

Age of heap	1 year
Place of heap	Previously used site Inside shed
Number of animals	40
Kind of animals	Sheep
Bedding used	Nothing
Fodder	Egyptian clover
Future use of heap	To sell
Portion used as fuel	Nothing
Quality of heap	good
Price of heap	£ 12.00

Heap 3 OWNER MR. GHULAM HUSSAIN

Age of heap	1 year
Place of heap	Previously used site out side shed
Number of animals	40
Kind of animals	Sheep
Bedding used	Nothing
Fodder	Egyptian clover
Future use of heap	To sell
Portion used as fuel	Nothing
Quality of heap	good
Sale price of heap	£ 12.00

HEAP 4 OWNER MR. AZIZ KHAN

Age of heap	2 days
Place of heap	Previously used site Out of doors
Number of animals	6
Kind of animals	4 cows, 1 goat and 1 lamb
Bedding used	Dried farmyard manure
Fodder	Egyptian clover plus barley
Future use of heap	As bedding
Portion used as fuel	50%
Quality of heap	Not good
Price of heap	Not for sale

HEAP 5 OWNER MR. JAN KHAN

Age of heap	2 years
Place of heap	Previously used site Out of doors
Number of animals	10
Kind of animals	1 camel, 2 bullocks, 4 cows 1 goat and 2 lambs
Bedding used	Dried farmyard manure
Fodder	Egyptian clover plus barley
Future use of heap	To sell
Portion used as fuel	50%
Quality of heap	Not good
Price of heap	£ 6.00

HEAP 6 FACULTY OF AGRICULTURE, GOMAL UNIVERSITY

Age of heap	6 months
Place of heap	Previously used site out of doors in ditch
Number of animals	12
Kind of animals	11 female and 1 male buffaloes
Bedding used	Nothing
Fodder	Egyptian clover plus barley green plus cotton cake and broken rice
Future use of heap	Spreading on land
Portion used as fuel	Nothing
Quality of heap	Good

The chemical composition of the above mentioned heaps are shown in Tables 6.5 to 6.7.

Table 6.5 Properties of farmyard manure heaps.

Heap. number	pH	Moisture (%)	Heap age (year)	Kind of animal
1	8.40	20.81	4	cattle
2	8.74	52.06	1	sheep
3	8.50	51.32	0.5	sheep
4	8.22	8.25	*	various
5	8.31	36.66	2	various
6	7.70	54.78	0.5	buffaloes

* 2 day old sun dried farmyard manure for use as bedding.

Table 6.5 shows that all heaps surveyed were alkaline. These heaps had different moisture contents. The heap 4 had the lowest moisture because it was a small sun dried heap of farmyard manure for use as bedding. The older heaps were found to be drier than new heaps.

The surveyed farmyard manure heaps were analysed for their available and total nitrogen, phosphorus and potassium. The plant available macronutrients are shown in Tables 6.6 and 6.7 while the total macronutrients are shown in Tables 6.8 and 6.9.

Table 6.6 Available nitrogen, phosphorus and potassium in farmyard manure heaps (fresh weight basis).

Heap number	Kind of animal	NH_4-N (mg/g)	NO_3-N (mg/g)	P (mg/g)	K (mg/g)
1	Cattle	0.38	0.15	0.65	11.1
2	Sheep	1.31	0.10	0.64	14.3
3	Sheep	0.55	0.11	0.91	11.3
4	Various	0.60	0.10	0.21	17.1
5	Various	0.22	0.08	0.71	7.75
6	Buffaloes	0.21	0.32	0.36	7.32

Table 6.7 Available nitrogen, phosphorus and potassium in farmyard manure heaps (oven dry weight basis).

Heap number	Kind of animal	NH_4-N (mg/g)	NO_3-N (mg/g)	P (mg/g)	K (mg/g)
1	Cattle	0.49	0.19	0.82	14.0
2	Sheep	2.73	0.21	1.34	29.8
3	Sheep	1.13	0.22	1.86	23.1
4	Various	0.65	0.11	0.23	18.6
5	Various	0.34	0.12	0.11	12.0
6	Buffaloes	0.46	0.71	0.80	16.2

Table 6.8 Total nitrogen, phosphorus and potassium in farmyard manure heaps (fresh weight basis).

Heap number	Kind of animal	N (%)	P (%)	K (%)
1	Cattle	0.91	0.21	1.70
2	Sheep	1.86	0.24	1.56
3	Sheep	1.30	0.25	1.39
4	Various	1.09	0.16	1.95
5	Various	0.87	0.19	1.42
6	Buffaloes	0.84	0.14	0.98

Table 6.9 Total nitrogen, phosphorus and potassium in farmyard manure heaps (oven dry weight basis).

Heap number	Kind of animal	N (%)	P (%)	K (%)
1	Cattle	1.16	0.28	2.16
2	Sheep	3.90	0.51	3.30
3	Sheep	2.65	0.52	2.98
4	Various	1.19	0.17	2.12
5	Various	1.35	0.29	2.20
6	Buffaloes	1.86	0.30	2.18

Table 6.10 Available nitrogen, phosphorus and potassium as a percent of total N, P and K.

Heap number	Kind of animal	N (%)	P (%)	K (%)
1	Cattle	6	31	65
2	Sheep	8	27	92
3	Sheep	5	36	81
4	Various	6	13	88
5	Various	3	37	55
6	Buffaloes	6	26	75

The results shown in Tables 6.6 and 6.7 reveal that animal type, heap age and storing method had effects on the available N, P and K levels. More available nutrients were present in farmyard manure produced from the sheep. The storage of farmyard manure stored in the open air and uncovered encouraged nitrogen loss and approximately one third of the nitrogen was lost compared with indoor heap. Among the farmyard manure produced from cattle, two day old sun dried farmyard manure for use as bedding showed more ammonium-N. This indicates that nitrogen is being lost from the older farmyard manure heaps. Nitrate-N was found to be greater in the farmyard

manure heap stored in a deep ditch which was produced from buffaloes housed at the Faculty of Agriculture. This might be due to its greater moisture content and the method of storage which reduced nitrogen loss, and encouraged nitrification compared with other heaps.

Available phosphorus was greater in sheep farmyard manure stored outdoor compared to indoor storage of farmyard manure. The reason might be greater breakdown of organic matter of the farmyard manure and mineralization of phosphorus.

The potassium results indicate that higher level of available potassium in the indoor stored farmyard manure heap produced from the sheep was due to less leaching by rain water. The fresh sun dried farmyard manure (heap no. 4) used as bedding had more available potassium than farmyard manures produced from cattle and mixed animals. This suggests that potassium may be lost by leaching.

41 and 78 % of nitrogen and potassium, respectively were lost from out door heap of sheep farmyard manure (heap 3) compared with farmyard manure stored indoor (heap 2).

The availability of nutrients as a percent of total N, P and K are shown in Table 6.10. These results revealed that available nitrogen is very low compared with phosphate and potassium availability. It means that much of nitrogen released as result of organic matter mineralisation has been lost.

The following points can be concluded from these results:-

Sheep produced farmyard manure richer in the major plant nutrients (nitrogen, phosphorus and potassium) than cattle.

Animal type, feeding and farmyard manure storage methods have effects on nutrient production and retention in farmyard manure.

Nutrients are lost from farmyard manure if piled out door and uncovered.

Loss of nutrients can be reduced by developing storing techniques for farmyard manure. Then these heaps can become more beneficial for farmers. Especially when synthetic fertilizers are in short supply in the market, the farmyard manure may be helpful in supplying plant nutrition and improving soil structure.

6.3.2 Experiment 2 (Nutrient Content of Farmyard manure)

This experiment was conducted to investigate the nitrogen, phosphorus and potassium contents in the fresh dung and urine of four buffaloes individually and also in their composite samples of dung and urine during summer and winter. In winter, only composite samples of dung and urine were analysed for determination of available nutrients. Total and available nitrogen, phosphorus and potassium were determined in the individual and composite samples during summer. The analysis results are shown in Tables 6.11 to 6.17.

Table 6.11 Chemical composition (total nutrients) of dung and urine of individual buffalo during summer (fresh weight basis).

Animal number	Moisture (%)	pH	N (mg/g)	P (µg/g)	K (mg/g)
Fresh dung					
1	84.6	7.8	1.96	634	0.82
2	84.9	8.1	1.89	427	0.88
3	84.0	8.0	1.71	327	0.73
4	84.3	8.0	1.52	549	0.82
Fresh urine					
1	-	8.1	3.16	8.3	8.70
2	-	8.7	1.15	7.4	8.13
3	-	8.1	1.03	9.2	9.69
4	-	7.8	1.35	13.8	11.07

All values are the mean of two replicates.

Table 6.12 Chemical composition (total nutrients) of dung and urine of composite samples from 4 buffaloes during summer (fresh weight basis).

Samples	Moisture (%)	pH	N (mg/g)	P (μg/g)	K (mg/g)
Fresh dung					
Day 1	82.5	8.0	1.09	394	0.87
Day 2	84.0	7.8	0.75	394	0.88
Day 3	82.0	8.0	1.33	346	0.88
Day 4	84.0	7.7	2.86	370	1.02
Day 5	83.0	7.8	1.36	479	0.90
Fresh urine					
Day 1	-	8.1	1.54	98.3	8.92
Day 2	-	8.0	1.41	39.0	11.68
Day 3	-	8.1	2.15	34.9	11.95
Day 4	-	8.1	3.61	18.3	11.95
Day 5	-	8.1	3.32	12.2	12.16

All values are the mean of two replicates.

Table 6.13 Available nutrients in dung and urine of individual buffalo during summer (fresh weight basis).

Animal number	NH_4-N (mg/g)	NO_3-N (mg/g)	P (μg/g)	K (mg/g)
Fresh dung				
1	0.40	ND*	334	0.71
2	0.42	ND	71	0.67
3	0.28	ND	242	0.58
4	0.33	ND	69	0.68
Fresh urine				
1	0.46	ND	4.3	4.85
2	0.37	ND	4.5	4.14
3	0.55	ND	3.7	5.35
4	0.83	ND	4.7	6.39

All values are the mean of two replicates.
* Not detectable (Less than 50 μg/g).

Table 6.14 Available nutrients in composite samples of dung and urine from 4 buffaloes during summer (fresh weight basis).

Samples	NH_4-N (mg/g)	NO_3-N (μg/g)	P (μg/g)	K (mg/g)
Fresh dung				
Day 1	0.19	ND*	251	0.75
Day 2	0.17	ND	219	0.79
Day 3	0.18	ND	212	0.87
Day 4	0.21	ND	209	0.80
Day 5	0.26	ND	189	0.75
Fresh urine				
Day 1	0.35	ND	2.7	5.02
Day 2	0.48	ND	4.1	5.60
Day 3	0.45	ND	2.1	6.18
Day 4	0.43	ND	3.5	5.10
Day 5	0.49	ND	5.7	5.59

All values are the mean of two replicates.
* Not detectable (less than 50 μg/g).

Table 6.15 Available nutrients (% of total) in the dung and urine of individual buffalo during summer (fresh weight basis).

Sample	Animal number	N (%)	P (%)	K (%)
Fresh dung				
	1	20	53	87
	2	22	16	76
	3	16	74	79
	4	22	13	83
Fresh urine				
	1	15	52	56
	2	32	61	51
	3	42	40	55
	4	61	34	58

Table 6.16 Available nutrients (% of total) in the composite samples from 4 buffaloes during summer (fresh weight basis).

	Sampling days	N (%)	P (%)	K (%)
Fresh dung				
	Day 1	17	64	86
	Day 2	23	56	86
	Day 3	14	61	99
	Day 4	7	56	78
	Day 5	19	39	83
Fresh urine				
	Day 1	23	3	56
	Day 2	34	11	48
	Day 3	21	6	52
	Day 4	12	19	48
	Day 5	15	47	46

Table 6.17 Chemical composition (available nutrients) of composite samples of dung and urine from 4 buffaloes during winter (fresh weight basis).

	Moisture (%)	pH	NH_4-N (mg/g)	P (µg/g)	K (mg/g)
Fresh dung					
Day 1	84.8	7.7	0.28	202	1.88
Day 2	83.9	7.3	0.29	200	1.91
Day 3	85.2	7.8	0.29	202	1.64
Day 4	85.2	7.8	0.29	198	1.59
Day 5	84.0	7.6	0.29	205	1.97
Day 6	84.0	7.8	0.28	202	1.95
Day 7	85.2	7.7	0.28	199	1.74
Day 8	85.2	7.7	0.28	202	1.88
Day 9	84.0	7.7	0.25	207	2.01
Day 10	83.1	7.8	0.27	203	1.81
Day 11	84.0	7.7	0.27	186	2.24
Day 12	84.0	7.8	0.29	200	1.95
Day 13	84.0	7.9	0.30	197	1.82
Day 14	86.6	7.7	0.26	205	1.66
Day 15	83.6	7.8	0.28	197	2.00
Fresh urine					
Day 1	-	7.6	0.23	44	9.06
Day 2	-	7.7	0.23	44	8.16
Day 3	-	7.8	0.24	32	8.71
Day 4	-	7.8	0.26	44	9.14
Day 5	-	7.8	0.24	44	8.25
Day 6	-	7.7	0.24	43	9.47
Day 7	-	7.7	0.22	44	8.93
Day 8	-	7.7	0.25	43	8.58
Day 9	-	7.6	0.21	43	9.23
Day 10	-	7.7	0.24	44	9.35
Day 11	-	7.8	0.22	44	9.59
Day 12	-	7.7	0.21	43	9.10
Day 13	-	7.8	0.24	44	9.35
Day 14	-	7.7	0.24	44	8.63
Day 15	-	7.7	0.22	45	9.57

All values are the mean of two replicates.

The results in Table 6.11 revealed that fresh dung and urine are alkaline in reaction. There were uniform amounts of nitrogen and potassium in the dung samples of the individual animals. Nitrogen and phosphorus contents were low compared to potassium in the urine. Nitrogen in

the urine of the milking buffalo (animal 1) was high. This might be due to its feed with cotton seed cake.

The chemical composition of the composite samples of dung and urine from a group of 4 buffaloes (Table 6.12) showed variability in nitrogen content of dung and urine samples analysed on five consecutive days. However, phosphorus and potassium contents were more uniform. Higher nitrogen and particularly potassium and lower phosphorus were found in urine than in the dung samples.

The study of Table 6.13 and 6.14 would reveal that individual animals are variable in excretiing available nitrogen, phosphorus and potassium in their fresh dung and urine. The composite samples collected and analysed from day to day showed reduced variability in available contents of nitrogen, phosphorus and potassium (see, Table 6.14). Nitrate was present in traces in the dung and urine of all four animals. Both dung and urine had low available phosphorus concentration but their available nitrogen and potassium contents were high. Comparatively urine contained more potassium than dung.

Table 6.14 shows the available nutrients present in the composite samples of dung and urine during summer feeding from a group of four buffaloes. It is clear from these results that production of available nitrogen, phosphorus and potassium was more uniform than their amounts determined in the samples from individual animals. Again, nitrate was present only in traces in the dung and urine. Similar to the analysis of individual samples, much lower phosphorus but higher nitrogen and potassium were

present in the urine than in the dung samples collected and analysed on five consecutive days of sampling.

The availability of nutrients as percentage of total nutrients (Tables 6.15 and 6.16) varied in the individual and composite samples of fresh dung and urine from individual buffalo. More nitrogen was in the available form in the urine than in the dung. Phosphorus was approximately equally available in both dung and urine samples. Potassium was more available in dung than urine.

The results presented in Table 6.17 show available nitrogen, phosphorus and potassium determined in the composite samples of dung and urine from same group of four buffaloes during winter feeding. More nitrogen than phosphorus was present in dung and urine. Dung and urine had almost the same available level of nitrogen whereas in summer it was different. Dung and urine produced from buffaloes during winter showed more available potassium than samples produced from same animals during summer. Urine nitrogen content was slight lower in winter samples than in the summer. The differences in nitrogen, phosphorus and potassium levels between summer and winter produced dung and urine seems to be due to the difference in feeding system. For example, green fodder during summer was forage maize (Zea mays L) while berseem (Trifolium alexandrinum L) mixed with wheat straw was fed to the animals during winter.

The pH results are in agreement with Richards and Wolton (1976) who reported that sheep urine was alkaline. The variation in phosphorus can be attributed to the age

and sex of animals as concluded by Simons and Jongbloed (1981) who reported that there is variation in the use of phosphorus consumed by animals of different ages and sexes, therefore, variation in phosphorus content of excreta varies from animal to animal. They also found that animals excrete only extra phosphorus surplus to their requirement. These results further agree with Maraikar and Amarasriri (1988) who found variation in nitrogen and phosphorus content in fresh dung of cattle but potassium was found to be more uniform. The variation in nitrogen of composite samples may be due to unequal contribution from the four animals in the composite samples. Nevertheless, the general composition was uniform from day to day.

The nitrogen, phosphorus and potassium contents found in fresh dung and urine are low in both individual samples and in composite samples compared to those reported by Manjunathaiah et al. (1989). They found the greatest available nitrogen concentration of 2.78% and available phosphorus concentration of 1.66% in pig dung while ass dung had the greatest available potassium content of 1.95%. They also reported low available phosphorus in goat, sheep, poultry and cow dung. They noted the greatest available magnesium in buffalo and pig dung.

The nitrogen content is lower than nitrogen content of cattle dung in the U.K which ranges from 0.32 to 0.52 % (Whitehead and Raistrick, 1993). In 1982, MAFF researchers summarised the total and available nutrients in animal farmyard manures and slurries. Comparison with their

results also indicate that farmyard manure produced from buffaloes is low in nitrogen, phosphorus and potassium. This difference of chemical composition is due to type of animals and their feeding system. In the U.K concentrates are included in feed of animals alongwith silage while in the Pakistani experimental conditions the fodder fed to animals was not even fertilized or organic farmyard manured. Therefore, dung and urine produced from the group of four buffaloes in summer or winter had low total and available nitrogen, phosphorus and potassium.

The following points can be concluded from the results described above for this experiment:-

Although individual and composite samples of fresh dung and urine were not collected at the same time during the summer they only differed slightly in their total and available nitrogen, phosphorus and potassium content.

Great variation was observed in the nutrients production among the animals. The chemical analysis of the composite samples of fresh dung and urine showed uniform level of nitrogen, phosphorus and potassium from day to day.

The differences in the level of nutrients in farmyard manure produced during summer and winter might be due to differences in the diet of animals.

On the basis of the above mentioned points, it is concluded that average chemical composition of farmyard manure will be the best estimate for the nutrients level if the fresh farmyard manure is to be applied to crops.

6.3.3 Experiment 3 (Production of Farmyard Manure)

This experiment was carried out to investigate the total farmyard manure production per buffalo per night and animals were tethered only during the night. They were loosed for grazing during the day. The other aim was to measure the major plant nutrients and to evaluate its fertilizer value.

Every morning, farmyard manure (faeces plus urine plus litter) was collected and weighed. The weight of sawdust used as litter was deducted. The data were analysed and the results are presented in Table 6.18.

Table 6.18 Overnight farmyard manure production by a group of four buffaloes.

	No bedding		Sawdust bedding	
	Summer manure (kg/animal)	drainage	Summer manure (kg/animal)	Winter manure (kg/animal)
Sample number	33	33	5	14
Mean	20	1	19	28
S.D.	2	2	5	5
Minimum	16	0	13	23
Maximum	24	11	25	43

No drainage resulted after using sawdust as bedding.

Overnight farmyard manure production varied between summer and winter seasons. This variation might be due to difference in the type of fodder fed to the animals during the two seasons. The other reasons seems to be longer nights of the winter and the less evaporation during the

winter. The amount of excreta varies with type and age of animals, housing and type of feed. The amount of excreta per dairy cow in the U.K.is 41 kg per day which is considered as the best estimate while the range is from 32 to 54 kg per cow per day (MAFF, 1982). Menzi et al. (1991) recorded 19 to 41 kg of slurry or 34 to 40 kg of fresh farmyard manure per cow per day under different farmyard manure handling system. In the present study, the range is from 23 to 43 kg per buffalo per night during winter while in summer it is lower which is from 13 to 25 kg per buffalo.

Table 6.19 Chemical composition of urine collected from the drain during summer (without bedding).

	pH (mg/g)	NH_4-N (mg/g)	NO_3-N (µg/g)	P (µg/g)	K (mg/g)
Day 1	9.0	4.12	63	19	19.1
Day 2	8.9	3.95	133	20	25.9
Day 3	8.8	4.64	ND	21	25.9
Day 4	8.8	1.52	56	19	22.5
Day 5	8.9	4.23	63	24	23.8
Day 6	8.9	3.51	ND	16	25.0

All values are mean of two replicates.
ND Not detectable (less than 50 µg/g).

The need to use bedding material was dictated by the chemical analysis of the drain water as shown in Table 6.19. High pH and ammonium nitrogen of this material indicated that there is more rapid transformation of urea nitrogen into the ammonical form. Phosphorus and nitrate were low as before in farmyard manure and fresh urine. Total nitrogen and potassium contents were very high in this liquid material compared to their level in fresh

urine (see, Tables 6.11 and 6.12). The proper reasons for high nitrogen and potassium may be high potassium concentration of urine, washing of materials from dung and yard surface by excreted urine, decomposition process refered to above and evaporation leading to concentrated urine and dust which could blow in the drain.

Table 6.20 Chemical composition (available nutrients) of farmyard manure produced overnight from four buffaloes.

	Moisture (%)	pH	NH_4-N (mg/g)	P (µg/g)	K (mg/g)
No bedding (summer)					
Day 1	77.8	8.2	0.82	352	1.64
Day 2	80.3	8.2	0.43	358	1.15
Day 3	81.5	8.2	0.37	263	1.04
Saw dust bedding (summer)					
Day 1	70.0	8.2	0.47	209	4.30
Day 2	65.0	8.2	0.66	230	5.94
Day 3	64.0	8.2	0.34	206	5.13
Day 4	64.0	7.9	0.34	137	5.23
Day 5	64.0	8.1	0.59	148	5.01
Saw dust bedding (winter)					
Day 1	75.6	8.2	0.66	349	5.27
Day 2	77.6	8.3	0.65	344	5.47
Day 3	75.1	8.2	0.68	340	4.93
Day 4	74.1	8.2	0.68	349	5.24
Day 5	75.5	8.2	0.67	356	5.64
Day 6	75.3	8.3	0.67	351	5.75
Day 7	75.2	8.2	0.66	346	5.34
Day 8	75.7	8.1	0.68	349	5.22
Day 9	76.0	8.3	0.67	350	4.77
Day 10	74.6	8.3	0.65	355	5.25
Day 11	73.3	8.2	0.65	335	5.66
Day 12	76.2	8.1	0.69	344	5.54
Day 13	75.0	8.1	0.79	342	4.74
Day 14	76.8	8.2	0.76	368	5.06
Day 15	74.1	8.2	0.67	378	5.81

Nitrate was not detectable (less than 50 µg/g).

The results of chemical analysis of farmyard manure produced overnight during summer and winter with or without use of sawdust bedding are shown in Table 6.10. The composition of a farmyard manure is variable, being dependent on the type of livestock, livestock diet, the method of handling and storage and the type and amount of litter. Although chemical analysis of a sample of farmyard manure or slurry will show the amounts of nutrients in the sample, the composition often varies between individual samples (MAFF,1982).

The moisture content decreased after using sawdust bedding. Similar results have been published by Sobel et al. (1988) who reported that addition of bedding to farmyard manure reduces the moisture content and improves the solid handling characteristics. The farmyard manure remained alkaline in reaction. The available nitrogen, phosphorus and potassium were slightly greater in farmyard manure produced during winter than summer. The first reason seems to be more farmyard manure production, the second may be a higher nutrient level in fodder and the third particularly for nitrogen may be a low night temperature and ultimately lower ammonia gas volatilization.

The effect of sawdust on nitrogen, phosphorus and potassium absorption is only clear in case of potassium. Four times more potassium remained in farmyard manure in summer and winter compared with no use of sawdust bedding in the summer experiment. It saved very little nitrogen while on phosphorus its effect is not clear. This is only

because of sawdust bedding absorbed greater amounts of the urine which contained a high potassium concentration.

The potential financial value of fresh farmyard manure was calculated from its available nutrient contents (see, Table 6.20). The cost of the nitrogen, phosphorus and potassium components of fertilizers in Pakistan (1992-93) were:-

N	21.7 p/kg
P	50.0 p/kg
K	13.0 p/kg

The potential financial value of the farmyard manure was calculated in two ways:-

1. The available nitrogen, phosphorus and potassium from Table 6.20 were taken and the value shown in Table 6.21 was calculated from the sum of the values of each nutrient as under:-

Value (£/tonne) = Average nutrient concentration (kg/tonne) x nutrient price (£/kg)

2. The value of the farmyard manure shown in Table 6.22 was calculated in pence per animal per night as follows:-

Value (p/animal/night) = Value of manure (p/kg) x manure production (kg/animal/night)

The results shown in Tables 6.21 and 6.22 idicate that the value of the farmyard manure is mainly due to its

potassium content. This is caused by using sawdust as bedding. This absorbed the urine which contained high levels of potassium. The nitrogen, phosphorus and potassium ratio of the farmyard manure is highly unbalanced. It will not supply fully the nitrogen and phosphorus required by different crops. Therefore, chemical fertilizers will have to be applied along with its application to maintain the nitrogen, phosphorus and potassium balance in the soil.

Table 6.21 Potential financial value of farmyard manure produced by buffaloes.

Type of farmyard manure		N	P	K	Total
			£/tonne		
SUMMER					
	No bedding	0.12	0.16	0.17	0.45
	Sawdust bedding	0.10	0.10	0.67	0.87
WINTER					
	Sawdust bedding	0.16	0.18	0.68	1.02

Table 6.22 Potential financial value of farmyard manure produce by buffaloes.

Type of farmyard manure		N	P	K	Total
			Pence/animal/night		
SUMMER					
	No bedding	0.24	0.32	0.34	0.90
	Sawdust bedding	0.26	0.26	1.74	2.26
WINTER					
	Sawdust bedding	0.56	0.63	2.38	3.57

The fertilizer value of the farmyard manure is only based on its soluble nutrients. This value will increase if total nitrogen, phosphorus and potassium are considered. The release of available nitrogen and phosphorus from organic matter by mineralization would also increase its fertilizer value. The nutrient balance will also be improved by release of mineralisable nitrogen and phosphorus. The above results show that the farmer can earn approximately thirteen pounds per animal annually. It will be worthwile to compare the economics of the use of the farmyard manure as fertilizer and as fuel material. Usually the dung is air dried and used as fuel in Dera Ismail Khan. The value of the farmyard manure as fuel is approximately twelve pounds per annum if a bag of dried dung is produced per animal per week. This is approximately equivalent to the value of the fertilizer value. But the selling price of a bag of dried dung bricks is variable. Its prices are more in villages near the urban areas than those located away from the cities. It seems important to point out that farmer does not fully benefit from the value of the farmyard manure when it is used as fuel. This is because of the differences in the social behaviour of the farmer's families towards the use of the farmyard manure. The majority of them are not willing to make dung bricks. The farmyard manure is dried and used or sold by those poorer families who have no animals. The owners of animal permit this in return for cleaning of the animal house or a small share of the benefit by selling the dried dung bricks. Some women make

dung bricks either for their own use or to sell to make some pocket money. All the fuel needed for heating and cooking is not met by the dung bricks, farmers have to buy or produce fuel wood to meet this demand.

The farmers perceptions of the fertilizer value of the farmyard manure is at a very low level, indicating that they regard it as waste material rather than a valuable plant nutrients source. This is so because the farmers do not know exactly about the chemical composition of the farmyard manure and its use as the fertilizer material. Research work about evaluation of the manure as a fertilizer value is lacking so the farmers are not utilizing the farmyard manure for improving soil physical properties and soil fertility.

Farmyard manure is not a suitable material for use as fuel due to the following demerits:-

(a) The women's health is at risk during making of the dung bricks due to lack of hygenic handling.

(b) The whole family health is at risk by inhaling the smoke during the burning of the dung bricks. Particularly, the babies and the children may suffer seriously.

(c) Food may be contaminated by the fine dung materials.

The benefits obtainable by using the farmyard manure as fertilizer can be briefly summarised below:-

In addition to the major nutrients, farmyard manures contain calcium, magnesium and various trace elements all of which help to maintain the levels of these essential

elements in the soil. Farmyard manure adds organic matter to the soil which can act as a soil conditioner and improve soil structure. Solid farmyard manure also stimulates biological activity in the soil. On fine sandy silty soils, increased structural stability can be important with small seeded crops. The emerging seedlings of such crops can suffer badly if the soil surface slakes under the influence of rain and irrigation and then sets into a hard crust when dry. The water holding capacity and drought resistance of both light and heavy soils can be increased by farmyard manure application. The provision of irrigation water in Dera Ismail Khan will provide an opportunity to use the farmyard manure as a fertilizer material. With the start of the water supply through the newly built Chasma right bank canal in the area, the farmers approach towards the use of the farmyard manure as fuel will be changed. They would like to use it as fertilizer for their crops.

Recommendations for future investigations

The above discussion indicates that farmers need to be told about the fertilizer value of the farmyard manure. In future, therefore, it will be imperative to carry out research projects focussed on the following objectives:-

Fertilizer value of the farmyard manure

Investigations can be carried out to improve the fertilizer value of the farmyard manure such as the use of bedding to prevent losses of nutrients through drainage.

Experiments may be conducted to prevent the nutrient losses during the farmyard manure storage.

Agronomic value of the farmyard manure

Field experiments may be established to investigate the effects of the farmyard manure application on the yield of crops. General improvement in soil fertility can also be investigated though less easily measured.

Extension of research findings

Research findings must be extended to the farmers either by publishing manuals for the efficient use of the farmyard manure or by establishing demonstration plots in the various villages.

Keeping in view the above mentioned objectives, a farmyard manure storage experiment has already been started. The objective of this experiment was to find out suitable storage methods for the prevention of the nutrient losses by volatilization and leaching.

6.3.4 Experiment 4 (Storage of Farmyard Manure)

This experiment was carried out to investigate the effect of various storage methods on the prevention of nutrient losses from the farmyard manure. The farmyard manure collected from buffaloes was stored in a ditch according to the following treatments:-

Open	uncovered.
Soil	soil covered.
Straw	wheat straw covered.
Plastic	plastic covered.

The soil layer was 15 cm in depth. The finely chopped wheat straw was spread in a layer 15 cm thick which was then covered by a soil layer of 5 cm in depth. The clear plastic sheet was spread in a double layer.

The farmyard manure sampled at various times during the storage period was analysed for its pH, moisture content, available nitrogen, phosphorus and potassium contents. The results are shown in Tables 6.23 to 6.25. The days with rain were noted during the storage period and are shown in Table 6.26.

The results shown in Table 6.23 reveal that farmyard manure was alkaline in reaction. The farmyard manures are generally alkaline in reaction. O'callaghan et al. (1971) reported that the pig slurries had pH from 8.0 to 8.5. Hoyle and Mattingly (1954) reported a decrease in the pH of a straw-sewage sludge compost from 7.5 to 5.7 due to the aeration. Flowers and Arnold (1983) determined pH of

the pig slurry which was 7.7. Paul and Beauchamp (1993) reported a pH of 8.5 and 8.4 in the solid beef cattle farmyard manure and composted farmyard manure, respectively. In the present study, the plastic covering increased the farmyard manure pH. This might be due to the accumulation of ammonia from the readily soluble ammonium produced by mineralization of the farmyard manure. Ammonia accumulated in this treatment due to reduced nitrification. On the other hand, the decrease in the pH of the farmyard manure stored under fully aerobic or partially aerobic conditions indicates the gaseous loss of ammonia nitrogen and nitrification which could result in the acidification of the farmyard manure.

The moisture content of the farmyard manure covered with plastic remained constant throughout the storage, but it fluctuated in other treatments. This variation in the moisture content may be related to the fluctuation in the atmospheric humidity, temperature and rainfall during the storage period. Cooke (1982) has compared the characteristics of cattle farmyard manure produced in the U.K. with the cattle farmyard of U.S.A. He reported an average moisture percentage of 76 and 79 for U.K. and U.S.A. cattle farmyard manures, respectively.

Table 6.23 pH and moisture content of farmyard manure stored by four methods.

	Age (days)	Open	Covered		
			Soil	Straw	Plastic
Moisture (%)	0	58	55	59	60
	29	38	43	50	73
	101	56	55	68	72
	131	37	52	63	70
	161	36	47	54	71
	191	32	51	53	70
	221	31	53	64	73
	230	42	58	60	69
	244	35	54	57	72
	292	34	40	60	66
pH	0	8.8	8.6	8.9	8.3
	29	8.4	8.4	8.5	9.0
	101	8.1	8.5	8.3	8.9
	131	8.0	8.1	7.7	8.8
	161	7.9	8.1	7.9	9.0
	191	7.8	8.0	7.7	8.7
	221	7.8	8.0	7.6	8.6
	230	8.0	7.9	7.7	8.8
	244	7.7	7.9	7.7	8.7
	292	8.1	7.9	8.2	8.9

All the values are mean of two replication.

Table 6.24 Available nitrogen in farmyard manure stored by four methods.

Age (days)	Open	Covered		
		Soil	Straw	Plastic
Ammonium-N (mg/g)				
0	0.66	0.66	0.71	0.59
29	0.26	0.25	0.31	1.43
101	0.12	0.18	0.17	0.83
131	0.10	0.15	0.17	0.51
161	0.09	0.12	0.14	0.31
191	0.03	0.03	0.05	0.16
221	0.14	0.19	0.23	0.42
230	0.06	0.04	0.03	0.05
244	0.06	0.07	0.15	0.17
292	0.10	0.08	0.19	0.32
Nitrate-N (mg/g)				
0	0.08	0.09	0.06	0.10
29	0.07	0.12	0.20	0.31
101	0.48	0.53	0.83	0.70
131	0.33	1.60	1.78	0.18
161	0.46	0.74	1.61	0.19
191	0.35	0.66	1.49	0.15
221	0.28	1.96	1.47	0.23
230	0.50	1.22	2.05	0.37
244	0.44	0.85	1.34	0.25
292	0.11	0.57	0.67	0.27
Ammonium plus nitrate (mg/g)				
0	0.74	0.75	0.77	0.69
29	0.33	0.37	0.51	1.74
101	0.60	0.71	1.0	1.53
131	0.43	0.75	1.95	0.69
161	0.55	0.86	1.75	0.50
191	0.38	0.69	1.54	0.31
221	0.42	2.15	1.70	0.65
230	0.56	1.26	2.08	0.42
244	0.50	0.92	1.49	0.42
292	0.21	0.65	0.86	0.59

All values are the mean of two replicates. These values are expressed in mg/g of oven dry farmyard manure.

The study of Table 6.24 reveals that these results are very variable. This might be due to sampling variability due to the heterogeous nature of the material. Alternatively it may be due to changes in the nutrient composition of the farmyard manure. The loss could be due

to gaseous losses as ammonia or by denitrification or leaching due to heavy periods of rain. The gain can be attributed to the mineralization of nitrogen and phosphorus from the organic fractions of the farmyard manure. Therefore, the results are discussed on the basis of general trends in the data and not on the specific data at individual sampling dates.

The results show that available nitrogen content fell in the farmyard manure exposed to the atmosphere during the 292 days of storage. 28.3 percent of the nitrogen was lost in this treatment. The farmyard manure covered with soil or straw showed an increase in available nitrogen compared with the initial levels of nitrogen in these treatments. This indicates the greater mineralization of nitrogen and phosphorus than their losses in these two treatments. However, the plastic covered farmyard manure retained a stable nitrogen content throughout storage. Decreases in ammonium and increases in nitrate contents of farmyard manure reflect the nitrification process in the farmyard manure during the storage period. The plastic covered treatment shows slow nitrification compared with the other treatments.

The results shown in Table 6.25 present clear evidence of leaching losses of potassium. Therefore, leaching losses of nitrate nitrogen are quite possible in the uncovered, soil covered and straw covered treatments. Gaseous ammonia loss may also have occured in these treatments. The low level of available nitrogen in the plastic covered treatment may also be due to

denitrification because of poor aeration caused by the plastic cover and the higher water content of the farmyard manure. Denitrification may also increase due to the high pH in this treatment. If mineralization rates of nitrogen are unaffected in the plastic covered farmyard manure then the nitrogen losses must be greater than in the farmyard manured covered by soil or straw. However, there is some evidence (discussed later) that phosphorus mineralization was reduced in the plastic treatment. The greater loss of nitrogen in the plastic covered treatment is unexpected since this was intended to reduce the nitrogen losses. The high potassium content in the plastic covered farmyard manure clearly shows that there is no leaching losses of potassium and therefore nitrate. Therefore, the conclusion is that the cause of the low available level of nitrogen is gaseous ammonia loss or denitrification.

With many systems of handling farmyard manure as solid, liquid or slurry and by composting, losses of nitrogen from the stored farmyard manure is a problem. Paul and Beauchamp (1989) reported that the aeration of dairy cattle farmyard manure slurry for four days decreased in content of volatile acids , resulting in a higher pH that favoured ammonium volatilization. O'Halloran, (1993) reported that aeration increased the ammonia volatilization in liquid hog farmyard manure. Gale et al. (1991) reported that ammonia volatilization from chicken farmyard manure was the cause of nitrogen decrease in the dried farmyard manure. Whitehead and Raistrick (1993) reported that when urine and dung are stored with

additional water as slurry the hydrolysis of urea nitrogen proceeds at least as rapidly as with urine alone. Mineralization of the dung organic matter occured more rapidly than with dung stored alone, but the particulate material of the dung immobilized some nitrogen from the urine. Yokoyama et al. (1991) reported that gaseous loss of the nitrogen due to ammonia volatilization are a problem for the efficient recycling of the nutrients in cow dung deposited on the soil surface. They measured gaseous loss of nitrogen from cow dung during four weeks and reported that it was almost unaffected by the activity of the dung beetle. The loss of gaseous nitrogen was the result primarily of ammonia volatilization. The literature published by MAFF (1982) shows that about 10 percent of the nitrogen may be lost to the air as nitrogen or ammonia gases, but the extent of these losses naturally depends on the method of handling and storage. If the farmyard manure is compressed firmly and remains undisturbed, for example, as in yarded cattle, little nitrogen is lost. If the farmyard manure is piled in a loose heap in layers as much as 40 percent of the nitrogen can be lost, especially if the heap is turned.

The present study showed that the plastic cover on the farmyard manure heap did not prevent efficiently the nitrogen losses as ammonia gas. Dewes et al. (1991). also found low level of available nitrogen in heaps of cattle farmyard manure protected from precipitation by plastic sheets. They determined that these sheets were insufficient for reducing both gaseous losses and liquid

leaching. Witter and Lopez-Real (1988) determined the nitrogen losses during the composting of sewage sludge-straw mixture. They reported that a cover of zeolite (clinoptilolite) and of a clay soil placed on top of the compost pile proved very effective in adsorbing the volatilized ammonia, whereas a layer of matured sewage sludge-straw compost proved to be ineffective. It can be concluded that soil or straw cover layers on the heaps of farmyard manure proved better than open or plastic sheeting for conserving the available nitrogen.

Table 6.25 Available phosphorus and potassium in farmyard manure stored by four methods.

Age (days)	Open	Covered		
		Soil	Straw	Plastic
Phosphate (mg/g)				
0	1.11	1.66	1.46	1.44
29	1.40	1.58	1.58	2.12
101	1.54	1.63	2.18	1.83
131	0.83	2.64	2.64	1.78
161	0.91	1.41	2.12	2.30
191	0.83	2.10	2.08	1.79
221	0.82	1.71	3.08	1.89
230	1.14	3.05	3.26	1.55
244	1.07	2.40	1.95	2.31
292	0.96	0.68	2.42	1.49
Potassium (mg/g)				
0	15.34	16.28	16.79	13.90
29	14.84	13.97	22.65	17.16
101	2.30	7.88	3.70	15.93
131	1.67	10.11	7.46	14.75
161	1.06	7.59	5.77	12.21
191	1.19	8.05	5.95	12.11
221	1.26	6.93	5.93	9.71
230	2.24	10.97	10.48	16.73
244	2.05	9.34	8.64	18.78
292	1.12	5.62	5.12	13.64

All values are the mean of two replicates. These values are expressed in mg/g of oven dry farmyard manure.

The available phosphorus in the stored farmyard manure determined at different times during storage showed evidence of phosphorus mineralization. The straw covered farmyard manure contained the greatest amounts of available phosphorus. The loss of available phosphorus from the farmyard manure left open in the ditch was greater than losses from partially or fully anaerobic storage conditions. The small loss of available phosphorus in exposed farmyard manure could be due to its loss by leaching. The straw and soil covered farmyard manures showed more available phosphorus than open and plastic covered farmyard manures. However, the general loss of available phosphorus in all methods of farmyard manure storage is less compared with the losses of nitrogen and potassium. The reason for high phosphorus in soil and straw covered farmyard manures seems to be the presence of suitable conditions for phosphorus mineralization and its low mobility. The low phosphorus content in the farmyard manure covered by the plastic compared with soil and straw covered might be slower phosphorus mineralization from the organic matter. The anaerobic conditions caused by the plastic cover could have resulted in reduced mineralization of phosphorus.

The initial level of the potassium in the farmyard manure was very high. The importance of leaching is evident from the results that potassium was the nutrient which was most affected adversely by leaching from the uncovered farmyard manure. Nearly all the available potassium was lost after 100 days of storage period. There

was also significant loss of potassium content of the farmyard manures either covered by soil or a straw layer. Thus the rain water was percolating through covers and caused leaching of the potassium. The plastic proved better than soil or straw covering because of preventing the rain water entry into the farmyard manure. The evidence for leaching losses in this study is clearly shown by the potassium levels.

The potassium fluctuation among the sampling dates may be partly attributed to rainy days during the storage periods. The potassium level in the exposed and partially covered farmyard manure decreased after every heavy rainfall such as between 29 and 101 days and between 244 and 292 days of storage. The leaching losses of the potassium has been reported by Archer, (1988). He described that when farmyard manure or broiler farmyard manure is stacked in the open, considerable nitrogen and potassium can be lost by leaching of rainwater through the heap. The nutrients losses have been published by MAFF (1982). When farmyard manure is stored in the open, up to 20 percent of the nitrogen, about 7 percent of the phosphorus and 35 percent of the potash are lost during the season by leaching.

Table 6.26 Days with significant rain during the storage period.

Sampling day number	Rainfall day number
0	--
29	--
	88
	89
	90
101	--
131	--
161	
	181
191	
	205
221	
	--
230	--
244	--
	246
	262
	263
	264
	265
	273
	274
292	--

Conclusions

1. The pH of the farmyard manure stored by the different methods ranged from 7.0 to 9.0. The anaerobic storage increased the farmyard manure pH while fully aerobic or partially aerobic stored farmyard manure lead to a decrease.

2. The nitrogen, phosphorus and potassium losses are great if the farmyard manure is left uncovered. The covering helps in reducing the nitrogen as gaseous losses and potassium leaching but does not prevent it.

3. The covering of the farmyard manure reduces the leaching losses. The soil or straw are not as effective as the plastic.

4. The plastic reduces leaching but increases the gaseous losses of the nitrogen.

5. The plastic covering reduces the mineralisation of nitrogen and phosphorus.

6 The soil and straw are, therefore, better than the plastic covering for the conservation of the nitrogen and phosphorus.

CHAPTER SEVEN

CONCLUSIONS

This thesis covers analytical aspects regarding the determination of ammonium-N by the Technicon AutoAnalyzer II and evaluation studies on farm animal wastes - algal pond effluent (Scottish Agricultural College, Auchincruive, Ayr) and farmyard manure (Pakistan) for their nitrogen, phosphorus and potassium contents.

Preliminary investigations regarding the interferences caused by amino acids in ammonium-N determination by using the Technicon AutoAnalyzer II showed that amino acids cause interferences in ammonium-N determination by the Berthelot indophenol colour reaction. The effect of reaction conditions such as reagent age, temperature and hypochlorite concentration indicated that freshly prepared reagents should be used in studies investigating interferences by amino acids. Room temperature should be stable or reagent bottles should be placed in a water bath having a constant temperature of 25°C. The hypochlorite reagent should be freshly prepared at a concentration of 50 ml l^{-1}.

Seventeen amino acids plus urea, galactosamine and glucosamine were evaluated for their interferences in ammonium-N determination in water, 2M potassium chloride and 0.5M potassium sulphate solutions at ammonium-N level of 0 and 1.0 mg l^{-1}. The results revealed that organic nitrogen compounds interfere both positively and

negatively in ammonium-N determination using the nitroprusside catalysed Berthelot reaction by the Technicon AutoAnalyzer II. Soil extracting solutions (2M potassium chloride and 0.5M potassium sulphate) further increased interferences by these organic compounds. These results suggest that a pre-treatment step either distillation or gas phase dialysis should be included to reduce the interferences caused by the amino acids in the ammonium-N determination by the Technicon AutoAnalyzer II.

The need to eliminate the interferences caused by organic compounds initiated the development of a dialysis system which could be included with the Technicon AutoAnalyzer II. The studies carried out for the development of a dialysis system showed that the ammonium recovery was inversely proportion to acceptor flow rate as shown in the Figures 3.3a and 3.3b (Chapter 3). The relationship between ammonia trapping and acceptor flow rate was considered a crucial parameter for further work and the best flow rate for donor and acceptor streams was chosen for the analysis of water and extract samples. The Technicon silicon gas dialysis membrane showed very low ammonia transference. Polytetrafluoroethene (PTFE) membranes allowed higher amounts of ammonia to be transferred through them. Recovery of ammonia using the standard 6 inch dialyser was 56.7%. Sample rates of 50, 40, 30 and 20 samples per hour were tested. Analysis at 20 samples per hour showed adequate peak separation, and good peak shapes. Mazumder (1992) examined the variability of

ammonium-N determination in a standard solution prepared in water (1 mg l^{-1}) in ten replicates using the AutoAnalyzer II without dialysis. The standard deviation of the results was between 0.002 and 0.004 for a 1 mg l^{-1} ammonium-N solution. Comparison of the present results with those of Mazumder, 1992 show that the present results were slightly less precise which is to be expected with the more complex system. Calibration curves are linear. It is, therefore, considered that the system is sufficiently precise and reliable for its use in ammonium-N determination.

The dialysis system was included in the main manifold for ammonium-N determination of the Technicon AutoAnalyzer II. Studies using this system indicated that only traces of ammonium-N were present as impurities in organic nitrogen compounds such as urea, aspartic acid, lysine and histidine. Positive and negative interferences in the ammonium-N determination were attributed to hydrolysis or participation of the organic nitrogen compounds in the colorimetric reaction. These positive and negative interferences were eliminated by dialysis. The level of random variability measured with dialysis was consistent with random variability due to solution preparation and to the system complexity. The dialysis system was further developed for the determination of ammonium-N in Kjeldahl digests.

The evaluation of the composition of algal pond effluent in relation to its ability to supply plant available nutrients was considered important before its

application to the pot experiment. A study on extraction of nitrogen, phosphorus and potassium from algal pond effluent comparing 0.1M hydrochloric acid, 0.05M sulphuric acid, 0.05M sodium sulphate and water as extractants alongwith a direct filtration of the effluent was conducted. The results of this study revealed that ammonium, phosphate and potassium should be measured in 0.1M hydrochloric acid extracts while nitrite and nitrate should be measured by direct filtration.

The results of the nutrients stability tests for the algal pond effluent stored at 2°C reveal that there were significant changes in ammonium and phosphate content of the effluent during storage. There were also variations in nitrite and nitrate levels although statistics could not be applied due to insufficient replication. Potassium levels in the effluent showed no significant change. The trend of change in the chemical composition of algal pond effluent suggests that it can be stored for a week at 2°C with only minor changes in ammonium, nitrite, nitrate, phosphate and potassium. It was considered that fresh or stored samples could be applied to the pot experiment but their analysis prior to each application would be essential.

The pot experiment was conducted under greenhouse conditions. The algal pond effluent was applied to ryegrass (Lolium perenne L.) for the evaluation of its manurial value and pollution potential. The results indicated that there was a good effect of the application of the algal pond effluent on the grass yield. Although it

can not compete with the standard fertilizer application in terms of ryegrass yield, pond effluent could partially replace fertilizer if applied as organic irrigation. There was a net release of nitrogen and phosphorus in the Darvel and the Dreghorn soils due to mineralisations of waste solids and soil organic matter. The level of residual nutrients at the end of the pot experiment was not greater than for the standard NPK fertilizer, therefore, it has no greater pollution hazard.

The volume to be applied to agricultural land can be controlled but the farmer will not know about its chemical composition. This will create difficulties in the application of these results to field conditions because the effluent studied was of a synthetic nature. Treated agricultural wastes such as algal pond effluent can be applied as organic irrigation but there is uncertainty about their nutrients concentration. The cost of its application also is another factor to be considered alongwith its pollution potential particularly from its soluble nitrogen content.

Experiments were carried out in Pakistan for the evaluation of farmyard manure for its nitrogen, phosphorus and potassium contents. A survey of farmyard manure heaps was made in the village of Lundah Sharif, Dera Ismail Khan, Pakistan. The analytical results of this experiment showed that farmyard manure produced by sheep was richer in the major plant nutrients (nitrogen, phosphorus and potassium) than cattle manure. Animal type, feeding and the farmyard manure storage methods have effects on

nutrient production and retention in the farmyard manure. The nutrients are lost from farmyard manure if it is piled out doors and uncovered. Loss of nutrients can be reduced by developing better storing techniques for the farmyard manure, so that these heaps can become more beneficial for farmers. Especially when synthetic fertilizers are in short supply in the market, farmyard manure may be helpful in supplying plant nutrition and improving soil structure.

The results obtained from the experiments conducted to investigate the nutrients content of the fresh dung and urine of buffaloes revealed that although individual and composite samples of fresh dung and urine were collected at different times during the summer, they only differed slightly in their total and available nitrogen, phosphorus and potassium contents. Greater variation was observed in the nutrient production among individual animals. The chemical analysis of the composite samples of fresh dung and urine showed a uniform level of nitrogen, phosphorus and potassium from day to day. The differences in the level of nutrients in farmyard manure produced during summer and winter might be due to differences in the diet of animals. The average chemical composition of farmyard manure would be the best estimate for the nutrient level if the fresh farmyard manure is to be applied to crops.

The range of the farmyard production is from 23 to 43 kg per buffalo per night during winter while in summer it is lower which is from 13 to 25 kg per buffalo per night. The effect of sawdust bedding on nitrogen, phosphorus and potassium absorption is only clear in case

of potassium. Four times more potassium remained in farmyard manure in summer and winter compared with no use of sawdust bedding in the summer experiment. It saved very little nitrogen while on phosphorus its effect is not clear. This is because of sawdust bedding absorbed greater amounts of urine which contained a high potassium concentration. Due to the low nutrient value and imbalance between nutrients, chemical fertilizers will have to be applied along with its application to maintain the nitrogen, phosphorus and potassium balance in the soil.

The results of storage experiments revealed that the pH of the farmyard manure stored by the different methods ranged from 7.0 to 9.0. Anaerobic storage increased the farmyard manure pH while fully aerobic or partially aerobic stored farmyard manure lead to a decrease. The nitrogen, phosphorus and potassium losses are great if the farmyard manure is left uncovered. The covering helps in reducing the gaseous losses of nitrogen and potassium leaching but does not prevent it. The soil or straw coverings were not as effective as plastic sheeting in preventing leaching losses. The plastic reduced leaching but increased the gaseous losses of the nitrogen. The plastic covering also reduced the mineralisations of nitrogen and phosphorus. The soil and straw coverings are, therefore, better than the plastic covering for the conservation of the nitrogen and phosphorus in the stored farmyard manure.

FURTHER DEVELOPMENT OF WORK IN PAKISTAN

The farmers in Pakistan need to be told about the fertilizer value of the farmyard manure. In future, therefore, it will be imperative to carry out research projects focussed on the following objectives:-

Fertilizer value of the farmyard manure

The present results were concerned about the production and nutrient level of the farmyard manure produced by buffaloes. It will be worthwhile to conduct further experimental work on farmyard manure produced by various kinds of animals. This work can be extended to different villages of different areas of Dera Ismail Khan.

Due to the heterogeneous nature of the material, the collection of sufficient data will be essential for taking the best estimate of nutrient level in the manure produced by different animals at different locations.

Investigations can be carried out to improve the fertilizer value of the farmyard manure such as the use of bedding to prevent losses of nutrients through drainage. Experiments may also be conducted to prevent nutrient losses during storage of the farmyard manure.

Agronomic value of the farmyard manure

Field experiments may be established to investigate the beneficial effects of the farmyard manure application on the yield of crops. General improvement in soil fertility can also be investigated though less easily measured.

Extension of research findings

Research findings must be extended to the farmers either by publishing manuals for the efficient use of the farmyard manure or by establishing demonstration plots in the various villages.

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