# Chapter 5
# Irrigation Water Quality


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**Abstract** The quality of irrigation waters differs in various regions, countries and locations based on how the groundwater has been extracted and used, the rainfall intensity and subsequent aquifer recharge. The use of groundwater for agriculture in hot arid countries where rainfall is scarce leads to increase groundwater salinity and limits the selection of crops for cultivation. It is therefore important to determine the irrigation water quality. The concentration and composition of soluble salts in water determines its quality for irrigation. Four basic criteria for evaluating water quality for irrigation purposes are described, including water salinity (EC), sodium hazard (sodium adsorption ratio-SAR), residual sodium carbonates (RSC) and ion toxicity. Toxicities of boron and chlorides to plants are described. More specifically the relative tolerance levels of plants to boron is tabulated for easy understanding. The most important part of this chapter is the modification of water quality diagram of US Salinity Laboratory Staff published in the year 1954, this diagram does not present EC over 2250 μS $cm^{-1}$, however, most of the irrigation waters present salinity levels higher than 2250 μS $cm^{-1}$. Therefore, to accommodate higher water salinity levels the water classification diagram is extended to water salinity of 30,000 μS $cm^{-1}$ allowing the users of the diagram to place EC values above 2250 μS $cm^{-1}$. The salinity and sodicity classes are included in this chapter to provide information for crop selection and develop salinity and sodicity management options. The procedures for water salinity reduction through blending of different waters and management of water sodicity using gypsum are described by giving examples.

**Keywords** Irrigation · Quality · Salinity · Sodicity · Boron · Chlorides · Toxicities · Blending · Gypsum requirement

## 1 Introduction

Water scarcity is seen as a major constraint to intensify agriculture in a sustainable manner as an attempt to meet the food requirements of a rapidly growing human population. The ever increasing human population, climate change due to increased emissions of greenhouse gases (GHGs), and intensification of agriculture, are putting



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severe pressure on the world's two major non-renewable resources of soil and water, and thus pose a big challenge to produce sufficient food to meet the current food demand. The present world population of 7.3 billion people is predicted to grow to over 9 billion by 2050, with the majority of this population increase occurring in developing countries, most of which already face food shortages. A 70% increase in current agricultural productivity will be required to produce sufficient food if these human population growth predictions prove to be correct. In this context, concerted efforts are being made globally to improve the effectiveness of water which will be used for enhancing the production of irrigated crops. Additionally, efforts are also being made to improve water harvesting and water conservation in rain-fed agriculture.

The injudicious use of saline/brackish water is all too often associated with the development of soil salinity, sodicity, ion toxicity, and groundwater pollution. Because of these negative effects, it is important to have a better understanding of exactly how the quality of water influences the management of irrigated agriculture, especially in arid and semi-arid regions.

Salinity, sodicity and ion toxicity are major problems in irrigation waters. In arid areas, where rainfall does not adequately leach salts from the soil, an accumulation of salts will occur in the crop's root-zone. Thus, periodic testing of soils and waters is required to monitor any change in salt content. Sodicity, the presence of excess sodium, will result in a deterioration of the soil structure, thereby reducing water penetration into and through the soil. Toxicity refers to the critical concentration of some salts such as chloride, boron, sodium and some trace elements, above which plant growth is adversely affected by those salts.

This chapter addresses several aspects of irrigation water quality and criteria to determine water quality. It will also cover management issues and soil responses to the use of irrigation water of varying quality. The information presented in this chapter is an updated and improved version of an excerpt from an earlier irrigation water quality manual (Shahid 2004).

## 2 Quality of Irrigation Water

The concentration and composition of soluble salts in water will determine its quality for various purposes (human and livestock drinking, irrigation of crops, etc.). The quality of water is, thus, an important component with regard to sustainable use of water for irrigated agriculture, especially when salinity development is expected to be a problem in an irrigated agricultural area.

There are four basic criteria for evaluating water quality for irrigation purposes:

- Total content of soluble salts (salinity hazard)
- Relative proportion of sodium ($Na^+$) to calcium ($Ca^{2+}$) and magnesium ($Mg^{2+}$) ions – sodium adsorption ratio (sodium hazard)

- Residual sodium carbonates (RSC) – bicarbonate ($HCO_3^-$) and carbonate ($CO_3^{2-}$) anions concentration, as it relates to $Ca^{2+}$ plus $Mg^{2+}$ ions.
- Excessive concentrations of elements that cause an ionic imbalance in plants or plant toxicity.

In order to achieve the first three important criteria, the following characteristics need to be determined in the irrigation waters: electrical conductivity (EC), soluble anions ($CO_3^{2-}$, $HCO_3^-$, $Cl^-$ and $SO_4^{2-}$) where $Cl^-$ and $SO_4^{2-}$ are optional and soluble cations ($Na^+$, $K^+$, $Ca^{2+}$, $Mg^{2+}$) where K is optional. Finally, boron level must also be measured. The pH of the irrigation water is not an acceptable criterion of water quality because the water pH tends to be buffered by the soil, and most crops can tolerate a wide pH range. A detailed description of the techniques commonly employed for the analysis of irrigation water is available (USSL Staff 1954; Bresler et al. 1982).

## 2.1 Salinity Hazard

Excess salt increases the osmotic pressure of the soil solution, a situation that can result in a physiological drought condition. Thus, even though the soil in the field appears to have plenty of moisture, the plants will wilt. This occurs because the plant roots are unable to take up soil-water due to its high osmotic potential. Thus, water lost from the plant shoot via transpiration cannot be replenished, and wilting occurs.

The total soluble salts (TSS) content of irrigation water is measured either by determining its electrical conductivity (EC), reported as micro Siemens per centimeter ($\mu S\ cm^{-1}$), or by determining the actual salt content in parts per million (ppm). Table 5.1 prescribes the guidelines for water use relative to its salt content.

**Table 5.1** Salinity hazard of irrigation water (Follett and Soltanpour 2002; Bauder et al. 2011)

| | Dissolved salt content | |
|---|---|---|
| Hazard | ppm | EC ($\mu S\ cm^{-1}$) |
| **None** – Water for which no detrimental effects will usually be noticed. | 500 | 750 |
| **Some** – Water that may have detrimental effects on sensitive crops. | 500–1000 | 750–1500 |
| **Moderate** – Water that may have adverse effects on many crops, thus requiring careful management practices. | 1000–2000 | 1500–3000 |
| **Severe** – Water that can be used for salt tolerant plants on permeable soils with careful management practices. | 2000–5000 | 3000–7500 |

### 2.1.1 Modified USSL Staff (1954) Water Salinity Classification

The USSL Staff (1954) water classification diagram does not present an EC over 2250 μS $cm^{-1}$. However, most of the water used for irrigation purposes possesses salinity levels which are higher than 2250 μS $cm^{-1}$. Therefore, in order to accommodate higher water salinity levels, Shahid and Mahmoudi (2014) have modified the USSL Staff (1954) water classification diagram by extending water salinity up to 30,000 μS $cm^{-1}$ (Fig. 5.1).

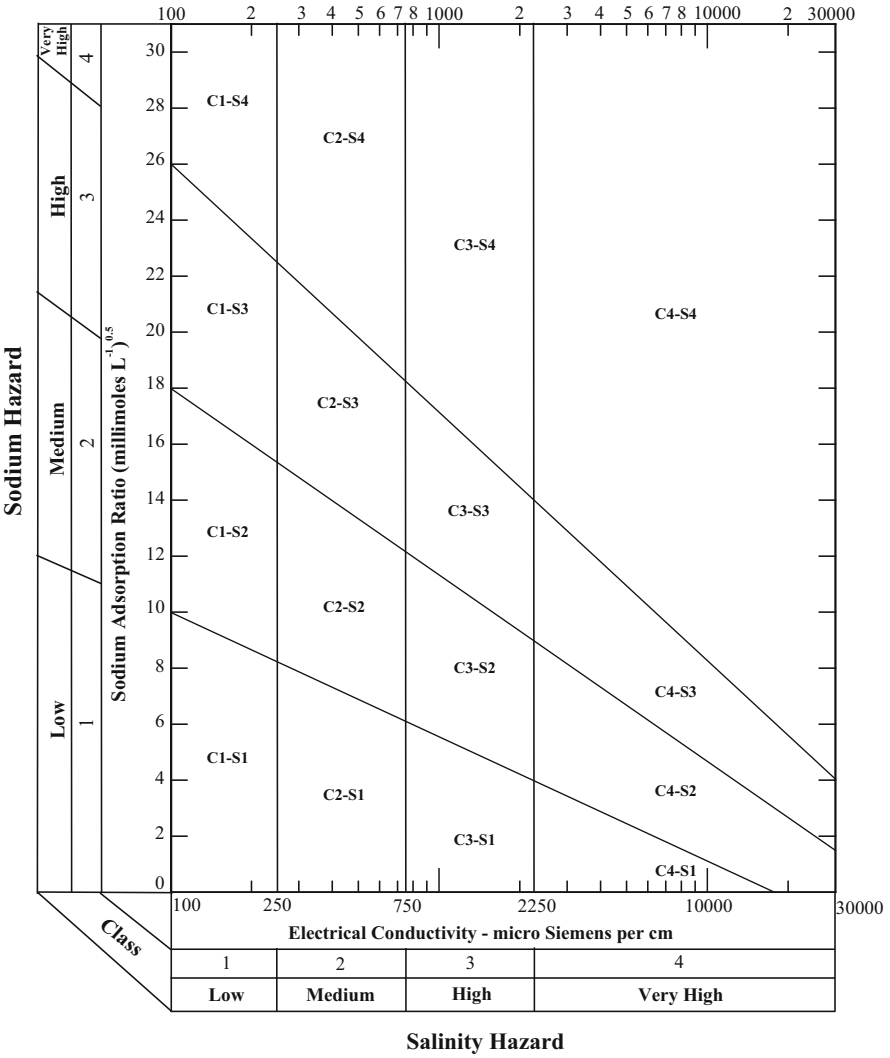


**Fig. 5.1** Diagram for the classification of irrigation waters (USSL Staff 1954; modified by Shahid and Mahmoudi 2014)

## 2.2 Sodium Hazard

The sodium hazard of irrigation water is expressed as the 'sodium adsorption ratio (SAR)'. Although sodium contributes directly to the total salinity and may also be toxic to sensitive crops, such as fruit trees, the main problem with a high sodium concentration is its effect on the physical properties of soil (soil structure degradation). It is, thus, recommended to avoid using water with an SAR value greater than 10 (mmoles $l^{-1})^{0.5}$, if the water will be the only source of irrigation for long periods.

This recommendation holds even if the total salt content is relatively low. For example, if the soil contains an appreciable amount of gypsum, SAR value of 10 (mmoles $l^{-1})^{0.5}$ can be exceeded. The gypsum content of the soil should, thus, be determined.

Continued use of water with a high SAR value leads to a breakdown in the physical structure of the soil – a situation caused by excessive amounts of adsorbed sodium on soil colloids. This breakdown in the soil physical structure, results in the dispersion of soil clay and that causes the soil to become hard and compact when dry, and increasingly impervious to water penetration (due to dispersion and swelling) when wet. Fine textured soils, those high in clay, are especially subject to this action. When the concentration of sodium becomes excessive (in proportion to calcium plus magnesium), the soil is said to be sodic. If calcium and magnesium are the predominant cations adsorbed onto the soil exchange complex, the soil can be easily tilled and will have a readily permeable granular structure.

The permissible value of the SAR is a function of salinity. High salinity levels reduce swelling and aggregate breakdown (dispersion), thus promoting water penetration. A high proportion of sodium, however, produces the opposite effect.

Regardless of the sodium content, water with an electrical conductivity (EC) less than about 200 μS $cm^{-1}$ causes degradation of the soil structure, promotes soil crusting and reduces water penetration. Rainfall is the prime example of low salinity water and rain water will reduce the penetration of water applied subsequently into soils. It is, thus, important that both the salinity and the sodium adsorption ratio of the applied water be considered when assessing the potential effects of water quality on water penetration into soils.

## 2.3 Carbonates and Bicarbonates Concentration

Waters high in carbonates ($CO_3^{2-}$) and bicarbonates ($HCO_3^-$) will tend to precipitate calcium carbonate ($CaCO_3$) and magnesium carbonate ($MgCO_3$), when the soil solution becomes concentrated through evapotranspiration. This means that the SAR

value will increase, and the relative proportion of sodium ions will become greater. This situation, in turn, will increase the sodium hazard of the soil-water to a level greater than indicated by the SAR value.

## 2.4 Specific Ion Effects (Toxic Elements)

In addition to salinity and sodium hazards, certain crops may be sensitive to the presence of moderate to high concentrations of specific ions in the irrigation waters or soil solution. Many trace elements are toxic to plants at very low concentrations. Both soil and water testing can help to discover any constituents that might be toxic. Direct toxicity to crops may result from some specific chemical elements in irrigation water, e.g. boron, chloride, and sodium are potentially toxic to plants. The actual concentration of an element in water that will cause toxic symptoms varies, depending on the crop.

When an element is added to the soil through irrigation, it may be inactivated by chemical reactions. Alternatively, it may buildup in the soil until it reaches a toxic level. An element at a given concentration in water may be immediately toxic to a crop. Or, it may require a number of years to accumulate in the soil before it becoming toxic.

### 2.4.1 Sodium Toxicity

Sodium toxicity can occur in the form of leaf burn, leaf scorch and dead tissues running along the outside edges of leaves. In contrast, $Cl^-$ toxicity is often seen at the extreme leaf tip. In tree crops, a sodium concentration (in excess of 0.25–0.5%) in the leaf tissue is often considered to be a toxic level of sodium. Correct diagnoses can be made from soil, water and plant tissue analysis.

Three levels of exchangeable sodium percentage (ESP) (FAO-UNESCO 1973; Pearson 1960; Abrol 1982), which correspond to three tolerance levels, are defined as: sensitive (ESP < 15), semi-tolerant (ESP 15–40) and tolerant (ESP > 40). The crops/plants listed as sensitive include, among others, beans, maize, peas, orange, peach, mung bean, mash, lentil, gram and cowpea. Semi-tolerant plants include carrot, clover, lettuce, berseem, oat, onion, radish, rye, sorghum, spinach, tomato, and tolerant plants include alfalfa, barley, beet, Rhoades grass and Karnal (Kallar) grass.

### 2.4.2 Boron Toxicity

Boron is essential to the normal growth of all plants, but the amount required is low. If it exceeds a certain level of tolerance depending on the crop, then boron may cause injury. The range between deficiency and toxicity of boron for many crops is narrow.

**Table 5.2** Effects of boron (B) concentration in irrigation water on crops (Follett and Soltanpour 2002; Bauder et al. 2011)

| Boron concentration (ppm) | Effect on crops |
|---|---|
| < 0.5 | Satisfactory for all crops |
| 0.5–1.0 | Satisfactory for most crops |
| 1.0–2.0 | Satisfactory for semi-tolerant crops |
| 2.0–4.0 | Satisfactory for tolerant crops only |

In order to sustain an adequate supply of boron to the plant at least 0.02 ppm of boron in the irrigation water may be required. However, to avoid toxicity, boron levels in irrigation water should, ideally, be lower than 0.3 ppm. Higher concentrations of boron will likely require that the intended crop type must first be evaluated with respect to its boron tolerance. Although boron toxicity is not a problem in most areas, it can be an important irrigation water quality parameter. Interestingly, plants grown in soils high in lime may tolerate higher levels of boron than those grown in non-calcareous soils.

Boron is weakly adsorbed by soils. Thus, its actual root-zone concentration may not vary in direct proportion to the degree that boron sourced from the irrigation water has been concentrated in the plant during growth. Symptoms of boron injury may include characteristic leaf 'burning', chlorosis and necrosis, although some boron sensitive species do not develop obvious symptoms. Boron toxicity symptoms first appear on older leaves as yellowing, spotting, or drying of leaf tissues at the tips and edges. The drying and chlorosis often progresses toward the center of the leaf, between the veins as boron accumulates over time (Ayers and Westcot 1985).

Irrigation water with boron >1.0 ppm may cause toxicity in boron sensitive crops. Table 5.2 describes the effects of a range of boron concentrations in irrigation water on crops (Bauder et al. 2011). The relative tolerance of plants to boron is shown in Table 5.3.

Boron levels that have developed in the soil water (saturation extract of soils) through irrigation can have a range of effects on crop yields. Wilcox (1960) presented three classes of crops with regard to boron toxicity: tolerant (2–4 ppm), semi-tolerant (1–2 ppm), and sensitive (0.3–1 ppm). Fruit crops are among the most boron sensitive, and yields of citrus and some stone fruit species are decreased by boron even at soil solution concentrations less than 0.5 ppm.

### 2.4.3 Chloride Toxicity

The most common crop toxicity is caused by chlorides in irrigation water. The chloride ($Cl^-$) anion occurs in all waters; chlorides are soluble and leach readily to drainage water. Chlorides are necessary for plant growth, though in high

**Table 5.3** Relative tolerance[a] of plants to Boron concentration (ppm) in irrigation water (cf. Ludwick et al. 1990; Ayers and Westcot 1985)

| Very sensitive < 0.5 ppm | Sensitive 0.5–0.75 ppm | Less sensitive 0.75–1.0 ppm | Moderately sensitive 1.0–2.0 ppm | Moderately tolerant 2.0–4.0 ppm | Tolerant 4.0–6.0 ppm | Very tolerant > 6.0 ppm |
|---|---|---|---|---|---|---|
| Lemon | Avocado | Garlic | Pepper, red | Lettuce | Tomato | Cotton |
| Blackberry | Grapefruit | Sweet potato | Pea | Cabbage | Parsley | Asparagus |
| | Orange | Sunflower | Carrot | Celery | Beet, red | |
| | Apricot | Bean | Radish | Turnip | | |
| | Peach | Sesame | Potato | Oats | | |
| | Cherry | Strawberry | Cucumber | Corn | | |
| | Plum | Bean, kidney | | Clover | | |
| | Grape | Peanut | | Squash | | |
| | Walnut | | | Muskmelon | | |
| | Onion | | | | | |

Adapted from 'Salt Tolerance of Plants' (Maas 1987), In: CRC Handbook of Plant Science in Agriculture

[a]Maximum concentrations tolerated in soil-water or saturation extract without yield or vegetative growth reduction. Boron tolerance varies depending upon climate, soil conditions and crop varieties. Maximum concentrations in the irrigation water are approximately equal to these values or slightly less

**Table 5.4** Chloride ($Cl^-$) levels of irrigation waters and their effects on crops (cf. Ludwick et al. 1990; Bauder et al. 2011)

| $Cl^-$ concentration | | |
|---|---|---|
| meq $l^{-1}$ | ppm | Effect on crops |
| < 2 | < 70 | Generally safe for all plants |
| 2–4 | 70–140 | Sensitive plants usually show slight to moderate injury |
| 4–10 | 141–350 | Moderately tolerant plants usually show slight to substantial injury |
| > 10 | > 350 | Can cause severe problems |

concentrations they can inhibit plant growth, and can be highly toxic to some plant species. Water must, thus, be analyzed for $Cl^-$ concentration when assessing water quality. Table 5.4 shows $Cl^-$ levels in irrigation water and the effects of $Cl^-$ on crops. In sensitive crops, symptoms occur when $Cl^-$ levels accumulate in leaves (0.3–1.0% on a dry weight basis). Ayers and Westcot (1985) reported that $Cl^-$ toxicity on plants appears first at the leaf tips (which is a very common symptom for chloride toxicity), and progresses from the leaf tip back along the edges as severity of the toxic effect increases. Excessive necrosis is often accompanied by early leaf drop or even total plant defoliation.

## 3 Classification of Irrigation Water

Shahid and Mahmoudi (2014) have modified the widely used USSL Staff (1954) salinity and sodium classification diagram for irrigation water (Fig. 5.1). This modified diagram is based on the EC (expressed in micro Siemens per cm – μS cm $^{-1}$) and the sodium adsorption ratio (SAR).

**How to Use the Diagram?**
The SAR as shown on y-axis (Fig. 5.1) can be calculated by using the following formula:

$$\mathrm{SAR} = \frac{\mathrm{Na}^{+}}{\sqrt{\frac{1}{2}\left(\mathrm{Ca}^{2+} + \mathrm{Mg}^{2+}\right)}}$$

Where, the concentrations of $Na^+$, $Ca^{2+}$ and $Mg^{2+}$ are expressed as milli equivalents per liter (meq $l^{-1}$). The values of the electrical conductivity given on the x-axis are expressed in micro Siemens per cm (μS $cm^{-1}$). The position of the SAR and EC points determines the quality class assigned to the water.

## 4 Analysis of Irrigation Water

### *4.1 Chemical Analyses*

The ultimate in water quality data for appraisal of salinity and sodicity includes complete analyses for all major cations and anions for both irrigation and drainage waters. Major cations normally include $Na^+$, $K^+$, $Ca^{2+}$ and $Mg^{2+}$. Major anions normally include $CO_3^{2-}$, $HCO_3^-$, $Cl^-$ and also $SO_4^{2-}$ (though see discussion below with regard to sulfate anion measurement).

When complete analyses are provided, it is possible to apply some simple tests for data consistency. For high quality water analysis, the sum of the cations in meq $l^{-1}$ should be approximately equal to the sum of anions in meq $l^{-1}$. If the values are exactly equal, however, for several water samples, this suggests that some constituents have been estimated by 'difference'. For example, recent analyses of sulfate have commonly been determined by difference because of the general unavailability of a rapid and convenient analytical procedure for measuring sulfate (Bresler et al. 1982). The $SO_4^{2-}$ estimation is based on the difference between total soluble cations and the sum of $CO_3^{2-}$, $HCO_3^-$, and $Cl^-$. In fact, sulfate is not a water constituent used to measure or determine either of SAR or Residual Sodium Carbonates (RSC). Thus, sulfate measurement currently has no assigned role in water quality assessment.

The data from above measurements are, thus, used to calculate the SAR in order to assess the sodicity hazard of the irrigation water, e.g. by use of Fig. 5.1 to obtain the water's sodicity (S) class. The EC, expressed in μS $cm^{-1}$, will then be used to obtain the conductivity (C) class of salinity. In addition, Residual Sodium Carbonate (RSC) can also be measured. These measurements are briefly described below.

### 4.1.1 EC and Total Salt Concentration

The most important water quality parameter from the standpoint of salinity is the total concentration of dissolved salts. It is different from 'total dissolved solids (TDS)', a term which carries some ambiguity. The measurement of TDS is much more tedious than measuring the EC – which is the preferred measure of salinity (Bresler et al. 1982). A simple meter is used to measure the electrical conductivity (EC) of both irrigation and drainage waters. Total salt concentration can then be obtained by using the following relationship for water having EC values between 0.1 and 10 milli Siemens per cm (mS $m^{-1}$) or dS $m^{-1}$ (Bresler et al. 1982):

$$\text{Total cations or anions } \left(\text{meq } l^{-1}\right) = 10 \times \text{EC } \left(\text{mS } cm^{-1} \text{or dS } m^{-1}\right)$$

Thus, once the concentrations of total cations or anions are known, the sum of cations or anions represents concentration of total salts contained within any solution.

### 4.1.2 Sodium Adsorption Ratio (SAR)

The tendency of salt solution to produce excessive exchangeable sodium in a soil must also be considered. A useful index for predicting this tendency is the Sodium Adsorption Ratio (SAR).

An SAR less than 8 (mmoles $l^{-1})^{0.5}$ is considered to be a 'low sodium' water class, i.e. the use of the irrigation water with SAR less than 8 is rated as being safe with regard to causing sodicity. That said, the prolonged use of class 8 SAR water for irrigation, when water drainage and leaching is restricted, may cause soils to develop sodicity. The detrimental effect of SAR also depends on the EC value, and in Pakistan an SAR of 10 is considered safe level (Kinje 1993).

**Adjusted SAR**

The significance of $SAR_{adj}$ is that under field conditions, and in normal conditions of irrigation management, the exchangeable sodium percentage (ESP) value in top soil is very nearly equal to the adjusted SAR, where pHc is calculated as the pH used in the Langelier Index of the irrigation water. Ayers and Westcot (1985) presented the term adjusted SAR ($SAR_{adj}$) as:

$$SAR_{adj} = SAR_{IW}[1 + (8.4 - pHc)]$$

The Langelier index is based on calculation of the pH which given water would achieve when in equilibrium with solid-phase calcium carbonates at average $CO_2$ values. This pH, when compared to the initial pH of the water, can be used to predict whether $CaCO_3$ should precipitate from or be dissolved by the waters as it passes through calcareous soil (Balba 1995). The pHc is the theoretical pH that water could have in equilibrium with $CaCO_3$.

### 4.1.3 Residual Sodium Carbonates (RSC)

There is another approach which is empirical in nature (Eaton 1950). It has been widely used to predict the additional sodium hazard which is associated with $CaCO_3$ and $MgCO_3$ precipitation, and involves a calculation of the residual sodium carbonates (RSC). This approach is based on the equation:

$$RSC\ (meq\ l^{-1}) = \left(CO_3^{2-} + HCO_3^{-}\right) - \left(Ca^{2+} + Mg^{2+}\right)$$

Where, all the concentrations are in meq $l^{-1}$. The ranges of RSC in meq $l^{-1}$ with respect to water suitability for irrigation are shown in Table 5.5.

## 5 Conductivity Classes (USSL Staff 1954)

There are four salinity classes, low, medium, high and very high, as presented in Table 5.6.

### *5.1 Low Salinity Water (Salinity Class C1)*

It can be used for irrigation of most crops on most soils with little likelihood that soil salinity will develop. Some leaching will be required for salinity Class C1 water, but

**Table 5.5** Residual sodium carbonates (RSC) and suitability of water for irrigation (Eaton 1950; Wilcox et al. 1954)

| RSC (meq $l^{-1}$) | Suitability of water for irrigation |
|---|---|
| < 1.25 | Safe |
| 1.25–2.50 | Marginal |
| > 2.5 | Unsuitable |

**Table 5.6** Salinity classes of irrigation waters (USSL Staff 1954)

| Salinity of irrigation water – EC (μS $cm^{-1}$) | Salinity class | Salinity hazard |
|---|---|---|
| 100–250 | C1 | Low |
| 250–750 | C2 | Medium |
| 750–2250 | C3 | High |
| > 2250 | C4 | Very high |

this occurs under normal irrigation practices, except for soils with extremely low permeability.

### *5.2 Medium Salinity Water (Salinity Class C2)*

It can be used if a moderate amount of leaching can occur. Plants with moderate salt tolerance can be grown in most cases without special practices for salinity control.

### *5.3 High Salinity Water (Salinity Class C3)*

It cannot be used on soils which possess restricted drainage and, thus, poor leaching abilities. Even with adequate drainage, special management for salinity control may be required and plants with good salt tolerance should always be selected.

### *5.4 Very High Salinity Water (Salinity Class C4)*

It is not suitable for irrigation under ordinary conditions, but may be used occasionally under very special circumstances. Here, the soils must be permeable, drainage must be adequate to good and irrigation water must be applied in excess in order to provide considerable leaching. Only very salt tolerant crops should be selected.

## 6 Sodicity Classes (USSL Staff 1954)

The classification of irrigation waters with respect to sodium adsorption ratio (SAR) is based primarily on the effects which exchangeable sodium accumulation has on the physical conditions of the soil. However, it should be kept in mind that sodium

sensitive plants may still suffer injury (as a result of sodium accumulation in plant tissues) even when exchangeable sodium values in soil-water are too low to bring about a deterioration of the physical condition of the soil.

## 6.1 Low Sodium Water (Sodicity Class S1)

It can be used for irrigation on almost all soils with little danger of the soil developing harmful levels of exchangeable sodium. However, sodium sensitive crops such as stone fruit trees and avocados may accumulate injurious concentrations of sodium.

## 6.2 Medium Sodium Water (Sodicity Class S2)

It will present an appreciable sodium hazard in fine textured soils which have high cation exchange capacity, especially under low leaching conditions, unless gypsum is present in the soil. Sodicity class S2 water may be used in coarse textured or organic soils with good permeability.

## 6.3 High Sodium Water (Sodicity Class S3)

It may produce harmful levels of exchangeable sodium in most soils. Its use will require special soil management methods, good drainage, a high leaching ability and high organic matter conditions. Gypsiferous soils, however, may not develop harmful levels of exchangeable sodium from such waters. Management methods may require use of chemical amendments which encourage the replacement of exchangeable sodium. That said, use of those amendments may not be feasible with waters of very high salinity.

## 6.4 Very High Sodium Water (Sodicity Class S4)

It is generally unsatisfactory for irrigation purposes except at low and perhaps medium salinity. Specifically, where the soil water solution is rich in calcium or the use of gypsum or other soil amendments may make the use of sodicity class S4 irrigation water feasible. Irrigation water sodicity classes and their hazards are given in Table 5.7.

**Table 5.7** Sodicity classes of irrigation water (USSL Staff 1954)

| SAR of irrigation water (mmoles $l^{-1})^{0.5}$ | Sodicity class | Sodicity hazard |
|---|---|---|
| < 10 | S1 | Low |
| 10–18 | S2 | Medium |
| 18–26 | S3 | High |
| > 26 | S4 | Very high |

Sometimes the irrigation water may dissolve sufficient calcium from calcareous soils to decrease the sodium hazard appreciably, and this should be taken into account using salinity class C1 – sodicity class S3 and salinity class C1 – sodicity class S4 irrigation waters. For calcareous soils with high pH values, or for non-calcareous soils, the sodium status of irrigation water in salinity class C1 – sodicity class S3, salinity class C1 – sodicity class S4, and salinity class C2 – sodicity class S4 may be improved by the addition of gypsum through lining of irrigation channels with gypsum stones or the sodium hazard may be countered by applying gypsum to the soil periodically. This is especially applicable when salinity class C2 – sodicity class S3 and salinity class C3 – sodicity class S2 irrigation water is used.

## 7 Improvement of Irrigation Water Quality

There are a number of ways to improve water quality, with regard to salinity and sodicity hazards, prior to using for irrigation purposes. Most commonly used practices are described below.

### *7.1 Blending Water*

The saline/brackish water quality can be improved if an alternate source of good quality water is available. The desired water salinity level, depending upon the crop to be irrigated, can be derived by a standard calculation procedure.

**Example**
A blend is made with 50% fresh water (EC 0.25 dS $m^{-1}$) with 50% brackish water (EC 3.9 dS $m^{-1}$). The resulting EC of the blended water would be:

$$\begin{aligned}&\text{EC(blended water)} = (\text{EC of fresh water} \times \text{mixing ratio}) + \\ &(\text{EC of brackish water} \times \text{mixing ratio}) = (0.25 \times 0.50) + (3.90 \times 0.50) \\ &= 0.125 + 1.95 = 2.075 \text{dS m}^{-1}\end{aligned}$$

## 7.2 Blending Water to Achieve a Desired Salinity

The desired water salinity can be achieved (by mixing two waters of known salinity) to irrigate a specific crop based on the threshold salinity. In this case, it is necessary to know what ratio of the two waters will be used to achieve the desired salinity.

**Example**
A blend is to be made of two waters, fresh (0.25 dS $m^{-1}$) with brackish (20 dS $m^{-1}$). Thus, we need to know 'in what ratio these two waters are to be mixed' to achieve a desired resultant water salinity of 8 dS $m^{-1}$.

Let us assume that we need to develop a final volume of 2 liters of the resultant water with a salinity of 8 dS $m^{-1}$.

$$\text{A standard formula can be used : } C1V1 = C2V2$$

Where,

C1 = 20 dS $m^{-1}$
V1 = unknown volume of the brackish water
C2 = 7.75 dS $m^{-1}$ or desired water salinity (8–0.25 = 7.75)
V2 = 2 liters or 2000 ml of desired final volume

Using the formula,

$$C1V1 = C2V2$$
$$20 \times V1 = 7.75 \times 2000 \text{ ml}$$
$$V1 = (7.75 \times 2000 \text{ ml})/20 = 775 \text{ ml}$$

Thus, 775 ml of the brackish water will be required to raise EC of the fresh water from 0.25 to 8 dS $m^{-1}$. The resulting blending ratio will be (1:2.58, i.e. the ratio of brackish water added to fresh water).

# 8 Water Sodicity Mitigation

Water sodicity can be mitigated through the judicious use of calcium-containing amendments such as gypsum ($CaSO_4.2H_2O$). Relative to other amendments, gypsum is cheap and easy to handle, and by far the most suitable amendment to bring down irrigation water sodicity (the ratio of sodium to calcium + magnesium). The quantity of gypsum needed for adding to irrigation water depends upon the quality of water (RSC and SAR levels) and the quantity of water required for irrigation during the growing season of the crop.

## 8.1 Gypsum Requirement Using the Residual Sodium Carbonates (RSC) Concept

**Example 1**
Irrigation water has an RSC 8.5 meq $l^{-1}$ and it needs to be reduced to 2.5 meq $l^{-1}$. The water required for irrigation is 800 mm per hectare for the complete growing period of the sorghum crop. How much gypsum will be required for adding to the water that is needed to irrigate one hectare, that water having the desired RSC of 2.5 meq $l^{-1}$?

- 1equivalent per liter of $Na^+$ will require 1 equivalent per liter of $Ca^{2+}$ which is equal to 86.06 grams of gypsum per liter of solution
- Therefore, 1 meq $l^{-1}$ of $Na^+$ will require 1 meq $l^{-1}$ of $Ca^{2+}$ which is equal to 0.08606 grams of gypsum per liter of solution
- Thus, 6 meq $l^{-1}$ of $Na^+$ will require 6 meq $l^{-1}$ of $Ca^{2+}$ which is equal to 0.51636 grams of gypsum per liter of solution
- Total water required to irrigate one hectare of sorghum crop = 800 mm × 10 = 8000 $M^3$ (Where, 1 mm of water in 1 hectare is equal to 10 $M^3$)
- 8000 $M^3$ of water is equal to 8000 × 1000 = 8,000,000 liters of irrigation water across the entire growing season
- Total gypsum requirement = 8,000,000 × 0.51636 = 4.13 metric tons of 100% pure gypsum
- If the gypsum purity is 70%, then 5.90 tons of gypsum will be required to neutralize 6 meq $l^{-1}$ of $Na^+$ in 8 million liters of irrigation water

To amend the water RSC, it is best to place the gypsum in the water channels. Then, the flowing irrigation water will dissolve the gypsum, reducing the $Na^+$:($Ca^{2+}$ + $Mg^{2+}$) ratio prior to entering the agricultural field.

**Example 2**
A farmer is using saline water with an EC of 3 dS $m^{-1}$ for irrigating a sorghum crop. He is facing problems with irrigation water infiltrating into his field soil and has decided to use gypsum. A laboratory analysis has shown that he needs an increase of 5 meq $l^{-1}$ of calcium in the irrigation water. How much gypsum would be required to irrigate one-hectare area with a crop water requirement for the entire growing period as 800 mm?

- EC of water = 3 dS $m^{-1}$
- Cropped area = 1 ha
- Gypsum purity = 70%

Total water requirement = 800 mm × 10 = 8000 $M^3$ = 8,000,000 liters.

- 1 meq $l^{-1}$ of $Na^+$ will require 1 meq $l^{-1}$ of $Ca^{2+}$ which is equal to 0.08606 grams of gypsum per liter of solution.

**Table 5.8** The chemical analyses of well water

| Water | EC dS $m^{-1}$ | Ion concentrations (meq $l^{-1}$) | | | | | | | | SAR (mmoles $l^{-1})^{0.5}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $CO_3^-$ | $HCO_3^{2-}$ | $Cl^-$ | $SO_4^{2-}$ | |
| Well water | 4 | 25 | 2 | 7 | 6 | 0 | 0 | 20 | 20 | 9.805 |
| Resultant water | 1 | 6.25 | 0.5 | 1.75 | 1.5 | 0 | 0 | 5 | 5 | 4.903 |

- 5 meq $l^{-1}$ of $Na^+$ will require 5 meq $l^{-1}$ of $Ca^{2+}$ which is equal to 0.4303 grams of gypsum per liter of solution.
- Total water required to irrigate one hectare of sorghum crop = 800 mm or 8000 $M^3$
- 8000 $M^3$ of water is equal to 8000 × 1000 = 8,000,000 liters.
- Total gypsum requirement = 8,000,000 × 0.4303 = 3.44 metric tons of 100% pure gypsum
- If gypsum purity is 70%, then 4.92 metric tons of gypsum will be required to neutralize 5 meq $l^{-1}$ of $Na^+$ in 8 million liters of water.

Thus, 4.91 tons of gypsum of about 10 mesh size (2 mm) will be required for the irrigation water application across the entire growing season.

## 8.2 *Determining the SAR of Blended Water to Be Used for Irrigation*

**Example 1**
Water from a well has the composition (Table 5.8) and this well water will be diluted in a1:3 ratio with desalinated water. What will be the resultant SAR of the blended water? Assume that the desalinated water has negligible EC and $Na^+$, $Ca^{2+}$, $Mg^{2+}$ contents.

After blending with a ratio of 1:3 (well water:desalinated water), the SAR of the resultant blended water is reduced to half. However, it should be noted that the EC is reduced to one-quarter of the well water. Therefore, care should be taken to understand such conversions.

**Example 2**
A canal water (EC = 1.0 dS $m^{-1}$) source is available to irrigate a crop. However, the volume of water is insufficient. The farmer has decided to blend well water with a ratio of 20% well water (5 dS $m^{-1}$) with 80% of canal water (1 dS $m^{-1}$). What will be the SAR of the resultant water? Following are the water analyses of canal, well and blend waters (Table 5.9).

**Table 5.9** The chemical analyses of the canal, well and the resultant (blended) waters

| Water | EC (dS $m^{-1}$) | Ion concentrations (meq $l^{-1}$) | | | | | | | | SAR (mmoles $l^{-1})^{0.5}$ |
|---|---|---|---|---|---|---|---|---|---|---|
| | | $Na^+$ | $K^+$ | $Ca^{2+}$ | $Mg^{2+}$ | $CO_3^{2-}$ | $HCO_3^-$ | $Cl^-$ | $SO_4^{2-}$ | |
| Canal water | 1.0 | 6.25 | 0.5 | 1.75 | 1.5 | 0 | 0 | 5.0 | 5.0 | 4.903 |
| Well water | 5.0 | 32.0 | 2.5 | 9.0 | 8.0 | 0 | 0 | 25.0 | 25.0 | 10.98 |
| Blended water | 1.8 | 11.4 | 0.9 | 3.2 | 2.8 | 0 | 0 | 9.0 | 9.0 | 6.58 |

Composition of blended water:

| | | | |
|---|---|---|---|
| EC | $= (1.0 \times 0.8) + (5.0 \times 0.20)$ | $= 0.8 + 1.0$ | $= 1.8$ dS $m^{-1}$ |
| $Ca^{2+}$ | $= (1.75 \times 0.8) + (9.0 \times 0.2)$ | $= 1.4 + 1.8$ | $= 3.2$ meq $l^{-1}$ |
| $Mg^{2+}$ | $= (1.5 \times 0.8) + (8 \times 0.2)$ | $= 1.2 + 1.6$ | $= 2.8$ meq $l^{-1}$ |
| $Na^+$ | $= (6.25 \times 0.8) + (32.0 \times 0.2)$ | $= 5.0 + 6.4$ | $= 11.4$ meq $l^{-1}$ |
| $K^+$ | $= (0.5 \times 0.80) + (2.5 \times 0.20)$ | $= 0.4 + 0.5$ | $= 0.9$ meq $l^{-1}$ |
| $Cl^-$ | $= (5.0 \times 0.80) + (25.0 \times 0.2)$ | $= 4.0 + 5.0$ | $= 9$ meq $l^{-1}$ |
| $SO_4^{2-}$ | $= (5.0 \times 0.80) + (25.0 \times 0.2)$ | $= 4.0 + 5.0$ | $= 9$ meq $l^{-1}$ |
| SAR | $= Na^+/[(Ca^{2+} + Mg^{2+})/2]^{0.5}$ | $= 11.4/[(3.2 + 2.8)/2]^{0.5}$ | $= 6.58$ (mmoles $l^{-1})^{0.5}$ |

Blending should, thus, be done with an objective. If the objective is to reduce SAR, but with the condition that adequate canal/fresh water is not available to irrigate the crop, then blending is desirable. If, however, a sufficient volume of canal water is available, then simply replacing well water with the canal's fresh water for irrigation is a good option. Other farm conditions must also be considered, e.g. infiltration problems due to high SAR. Addition of gypsum as described above should also be considered.

## 9 Cyclic Use of Water

Where fresh water is also available, but not sufficient to offset the full water requirement of the crop, there is always a need to find alternate source of water, which is usually the groundwater and is often saline or saline-sodic. Under such conditions, it is recommended to use fresh water at early stage of crop when the young seedlings are not able to tolerate high salinity level. Once the seedlings are well established, at this stage there are two options to use these waters: (i) to use saline water for some time and then leach the salts with fresh water, and (ii) use saline water first and then use fresh water (cyclic use) to irrigate the crop. This way both fresh and saline waters are used.

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# Chapter 6
# The Role of Nuclear Techniques in Biosaline Agriculture


**Mohammad Zaman, Shabbir A. Shahid, and Lee Heng**

**Abstract** The major constraints under Saline Agriculture are the availability of essential nutrients and water to the plant which are adversely affected by excessive salts in the soil solution. Among the essential plant nutrients, N plays a key role in plant growth and productivity. Nuclear and isotopic techniques (also called nuclear-based techniques) are a complement to, not a substitute for, non-nuclear conventional techniques. Nuclear-based techniques, however, do have several advantages over conventional techniques by providing unique, precise and quantitative data on soil nutrient and soil moisture pools and fluxes in the soil-plant-water and atmosphere systems. Isotopic techniques provide useful information in assessing soil-water-nutrient management which can be tailored to specific agroecosystems for managing soil salinity. For example, $^{15}N$ stable isotopic techniques can be used to measure rates of the various N transformation processes in soil-plant-water and atmosphere systems, such as N mineralization-immobilization, nitrification, biological $N_2$ fixation, N use efficiency, and microbial sources of production of nitrous oxide ($N_2O$), a greenhouse and ozone depleting gas, in soil. The use of oxygen-18, hydrogen-2 (deuterium) and other isotopes is an integral part of agricultural water management, allowing the identification of water sources and the tracking of water movement and pathways within agricultural landscapes as influenced by different irrigation technologies, cropping systems and farming practices. It also helps in the understanding of plant water use, quantifying crop transpiration and soil evaporation and allows us to devise strategies to improve crop production, reduce unproductive water losses and prevent land and water degradation.

**Keywords** Isotopic and nuclear techniques · N-15 · Oxygen-18 · Hydrogen-2 · Salinity



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## 1 Introduction

Among the numerous abiotic and biotic stresses that affect plant productivity worldwide, soil water stress (drought) is the most common growth limiting factor in arid and semi-arid regions (Saranga et al. 2001), followed closely by salt stress (Pessarakli 1991). Development of a sustainable agriculture will require the combined use of soil, nutrient, and water management strategies that enhance crop productivity, while at the same time reducing abiotic and biotic stresses. To reach a truly sustainable agriculture, new 'climate smart' agricultural practices will need to be developed and adopted by the end users. These climate smart practices include both management strategies and specific technologies, ones which enhance crop productivity, environmental sustainability and wise use (conservation) of agro-ecosystems.

The Soil and Water Management & Crop Nutrition (SWMCN) subprogram of the Joint Food and Agriculture Organization (FAO) and International Atomic Energy Agency (IAEA)'s Division of Nuclear Applications in Food and Agriculture, has developed a wide range of nuclear and isotopic techniques to enhance nutrient and water use efficiencies, increase biological N fixation through the capture of atmospheric di-nitrogen ($N_2$) and carbon (C) storage in salt affected soil.

## 2 Background Information on Isotopes

The number of protons plus neutrons present in the nucleus of an atom is called the atomic weight, while the number of protons (or electrons – which is always equal) is known as atomic number. Isotopes are defined as atoms of the same atomic number but differing atomic weight. For example, nitrogen (N) has one isotope ($^{15}N$), which has the same number of protons (7) as $^{14}N$, but one extra neutron. This gives it ($^{15}N$) a different atomic weight ($7 + 8 = 15$).

Isotopes may exist in both stable and unstable (radioactive) forms, depending on the stability of the nucleus in an atom. For example, the sulfur (S) consists of 5 isotopes ($^{32}S$, $^{33}S$, $^{34}S$, $^{35}S$ and $^{36}S$); one of which ($^{35}S$) is a radioactive beta emitter, while the other four ($^{32}S$, $^{33}S$, $^{34}S$ and $^{36}S$) are stable. Thus, a radioactive isotope is an atom with an unstable nucleus which spontaneously emits radiation (alpha or beta particles and/or gamma electromagnetic rays). The non-stability occurs because the ratio of neutrons to protons in a nucleus lies outside the belt of stability (i.e., outside a particular number due to an excess of either protons or neutrons), which varies with each atom. In contrast, a stable isotope is an atom with a stable nucleus (i.e., the ratio of neutrons to protons in the nucleus of an atom is within the belt of stability), and hence, it does not spontaneously emit any radiation (Nguyen et al. 2011). Stable isotopes exist in light and heavy forms with heavy isotopes having a higher atomic weight than light isotopes (Table 6.1).

**Table 6.1** Average abundances of stable isotopes (% abundance in brackets) of some of the major elements commonly occurring in agro-ecosystems

| Element | Heavy isotope | Light isotope |
|---|---|---|
| Carbon | $^{13}C$ (1.108%) | $^{12}C$ (98.892%) |
| Hydrogen | $^{2}H$ (0.0156%) | $^{1}H$ (99.984%) |
| Nitrogen | $^{15}N$ (0.366%) | $^{14}N$ (99.634%) |
| Oxygen | $^{18}O$ (0.204%) | $^{16}O$ (99.759%) |
| | $^{17}O$ (0.037%) | |
| Sulfur | $^{33}S$ (0.76%) | $^{32}S$ (95.02%) |
| | $^{34}S$ (4.22%) | |
| | $^{36}S$ (0.02%) | |

The quantity of a stable isotope is measured by an Elemental Analyser coupled to an Isotope Ratio Mass Spectrometer (IRMS). Thus, a sample of soil or biological material is combusted into a gas, which is fed into a mass spectrometer, where the ratio of the stable isotopes of interest (e.g., $^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{15}N/^{14}N$, $^{18}O/^{16}O$,$^{33}S/^{32}S$) is determined.

Radioactive isotopes (radioisotopes) are measured by their rate of 'decay', e.g. liquid scintillation counters are used for beta particle emitting radioactive isotopes, gamma spectrometers for gamma ray emitting radioactive isotopes and alpha spectrometers for alpha particle emitting radioactive isotopes. The international unit (SI) of activity decay is the Becquerel (Bq), which is equal to one disintegration per second (dps). The old unit commonly used was called the Curie, which is equivalent to $3.7 \times 10^{10}$ dps or $3.7 \times 10^{10}$ Bq (Nguyen et al. 2011).

## 3 Use of Nuclear and Isotopic Techniques in Biosaline Agriculture

Nuclear and isotopic techniques (also called nuclear-based techniques) are a complement to, not a substitute for, non-nuclear conventional techniques. Nuclear-based techniques, however, do have several advantages over conventional techniques by providing unique, precise and quantitative data on soil nutrient and soil moisture pools and fluxes in the soil-plant-water and atmosphere systems. Isotopic techniques provide useful information in assessing soil-water-nutrient management which can be tailored to specific agro-ecosystems for managing soil salinity. For example, $^{15}N$ stable isotopic techniques can be used to measure rates of the various N transformation processes in soil-plant-water and atmosphere systems, such as N mineralization-immobilization, nitrification, biological $N_2$ fixation, N use efficiency, and microbial sources of production of nitrous oxide ($N_2O$), a greenhouse and ozone depleting gas, in soil. Several nuclear and isotopic techniques are being employed in soil water management studies. The soil moisture neutron probe is ideal in field-scale rooting zone measurement of soil water, providing accurate data on the availability of water for determining crop water use and water use efficiency and for establishing optimal

irrigation scheduling under different cropping systems especially under saline conditions.

The use of oxygen-18, hydrogen-2 (deuterium) and other isotopes is an integral part of agricultural water management, allowing the identification of water sources and the tracking of water movement and pathways within agricultural landscapes as influenced by different irrigation technologies, cropping systems and farming practices. It also helps in the understanding of plant water use, quantifying crop transpiration and soil evaporation and allows us to devise strategies to improve crop production, reduce unproductive water losses and prevent land and water degradation.

For details on the principles and applications of the various nuclear and isotopic techniques in soil, water and plant nutrient studies in agro-ecosystems, the readers are referred to the IAEA Training Manuals (IAEA 1990, 2001) and the review paper published by Nguyen et al. (2011). In below section, a stepwise protocol has been described to set up a field study to quantify fertilizer use efficiency of the added fertilizer.

## 4 The Use of Nitrogen-15 ($^{15}N$) to Study Fertilizer Use Efficiency

The major constraints under *Saline Agriculture* are the availability of essential nutrients and water to the plant which are adversely affected by excessive salts in the soil solution. Among the essential plant nutrients, N plays a key role in plant growth and productivity. To take up N from the soil solution, plants compete with a range of N removal processes/losses including immobilization, leaching, and gaseous emissions of N as ammonia ($NH_3$), nitrous oxide ($N_2O$), nitric oxide (NO) and molecular nitrogen ($N_2$) into the atmosphere. Because of these N losses, the N use efficiency (kg of dry matter produced per kg of N applied) or useful use of N by plant is invariably less than 50% of the applied N (Zaman et al. 2013a, b, 2014). The extent to which N is removed from soils, or made unavailable to plants by the above biogeochemical processes is of both economic and environmental importance.

Under saline conditions, the presence of excessive salts (especially $Na^+$) in the soil solution, coupled with a high soil pH, is likely to further increase the competition between N uptake by the plant and the soil N losses, thereby reducing crop productivity further. Quantifying N use efficiency and the sources of N losses enables researchers to develop 'technology packages' which can enhance N uptake and minimize N losses, thus allowing for sustainable crop productivity under saline conditions.

**Fig. 6.1** A wheat trial set up on a flat soil

## *4.1 Setting Up Experimental Field Plots*

In order to determine the N fertilizer use efficiency (NUE) of a wheat crop with a high degree of accuracy, a researcher shall set up a field trial on a relatively flat site with uniform fertility and slope so as to minimize background variations of soil nutrient levels, especially N and nutrients losses via surface runoff (Fig. 6.1).

Considering an experimental trial of N fertilizer applied at four rates: zero or control (T1), low (T2), middle (T3), and high (T4) of kg N per ha, with four individual replicate plots (each plot being 7 m × 7 m) for each of the four rates of N. (see schematic diagram below – Fig. 6.2).

A 'buffer zone' of 2 m wide on each of the four sides of the experimental site, with a 2 m wide strip between each of the individual replicate plots is especially important to prevent contamination of adjacent plots by N via surface runoff after heavy irrigation or rainfall, as well as lateral movement of N within the soil. The individual (replicate) field plots can be a range of sizes, depending on available land area, experimental design, farm resources (machinery) and most importantly available budget. Generally, a larger size for each individual replicate plot (e.g., 7 m long × 7 m wide) is considered as the best for minimizing edge effects (nutrient losses from the fertilized area to an un-fertilized area) on final crop yield, with each of four replicate plots being placed within four different treatment blocks.

- Prior to treatment application, four composite soil samples (each composite soil sample consist of ten soil cores from each experimental block) from 0–15 cm depth, shall be collected to analyze for key soil properties including, soil pH, ECe, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, $K^+$, total N, total C, and Olsen P.

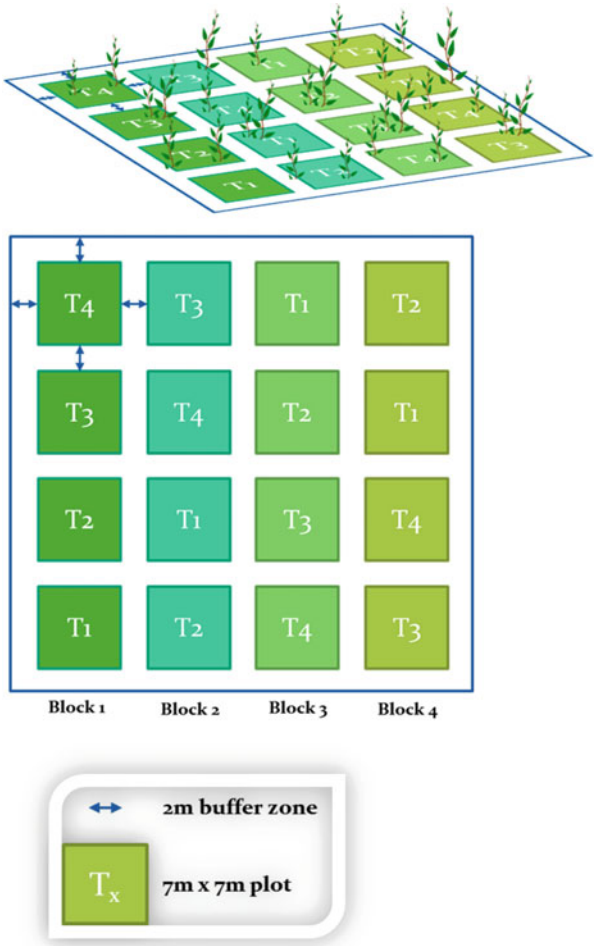


**Fig. 6.2** A schematic diagram of experimental layout

- First apply any soil amendments such as gypsum, and other chemical fertilizers without N (P and K as recommended) and animal manure.
- Assuming 7 m × 7 m (49 $m^2$) replicated field plot receiving N-fertilizer in the form of granular urea (46%N) at rate of 80 kg N $ha^{-1}$ in two split applications during wheat growth period, the amount of urea is calculated below:

$$\text{Rate of fertilizer application (kg per ha)} = \frac{100 \times \text{nutrient element required (kg per ha)}}{\%\text{nutrient element concentration in a fertilizer.}}$$

**Example:**
The amount of urea for the first application (40 kg N $ha^{-1}$) can be calculated as.

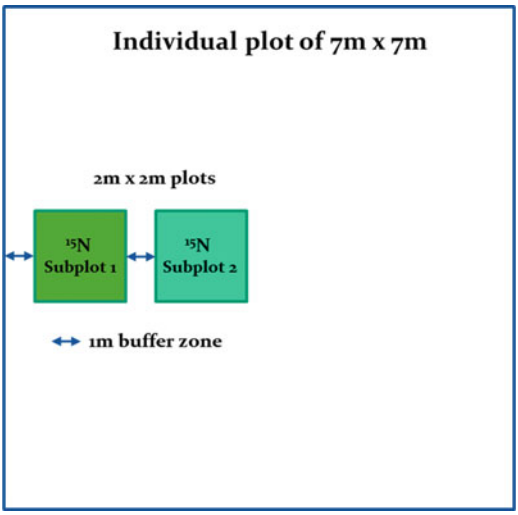


**Fig. 6.3** Schematic diagram of the layout of the two sub-plots within a main plot, each with a 1 m buffer zone, each destined for $^{15}$N-labeled fertilizer application

$$\text{kg of urea required per ha for the first application} = \frac{100 \times 40}{46} = 86.95 \text{ kg urea} \tag{6.1}$$

As mentioned below, during the N fertilization, one sub-plot (4 $m^2$) of $^{15}$N labeled urea within the 49 $m^2$ replicated plot will not receive ordinary urea. This leaves 45 $m^2$ area (49 minus 4) which will receive ordinary urea. Thus at 40 kg N $ha^{-1}$ rate, the amount of urea for 45 $m^2$ is calculated as:

$$\text{Amount of urea for 45 m}^2 = \frac{86.95 \text{ kg urea}}{10\,000 \text{ m}^2} \times 45 \text{ m}^2 = 0.39 \text{ kg} \tag{6.2}$$

Where, 10,000 $m^2$ correspond to the land area of one hectare.

**Setting up Sub-Plot for $^{15}$N Labelled Fertilizer:**

- For two split applications of $^{15}$N-labelled urea, one shall set up two sub-plots, each of 2 m × 2 m (4 $m^2$), separated within the entire 49 $m^2$ larger replicated plot by a 1 m buffer zone, as shown below (Fig. 6.3). This 4 $m^2$ sub-plot will allow researcher to select a few wheat plants for $^{15}$N analysis. The buffer zone will also help to minimize $^{15}$N contamination from adjacent sub-plot.

  [Mark each sub-plot well to avoid any mistake of fertilizer application].

- To ensure that no $^{15}$N-labeled fertilizer/residues are present from previous experiments, collect four soil cores (0–10 cm soil depth) from each of the two sub-plots, then combine them into one sample, and analyze for $^{15}$N content. This will establish the initial $^{15}$N level in the soil.

- Calculate the amount of $^{15}$N-labeled fertilizer (using a maximum of 5 atom % excess) to add to each **4** m$^2$ sub-plot using Eqs. 6.1 and 6.2. The amount of $^{15}$N-labeled urea at 40 kg N ha$^{-1}$ for a 4 m$^2$ sub-plot comes out to be 34.78 gram.
- Please note that if N fertilizer is applied in a single application, this 5 atom % excess could be reduced to 3 atom % excess (please refer to the dilution procedure at the end of this section).
- Separate the first sub-plot for $^{15}$N-labeled fertilizer by placing a temporary plastic sheet or any other similar material around the perimeter of the first sub-plot. Then, uniformly apply the required amount (0.39 kg) of ordinary urea to the entire (45 m$^2$) of the larger main plot excluding the first sub-plot.
- After application of the ordinary urea, remove the plastic sheet around the first sub-plot, carefully weigh out the exact amount of $^{15}$N-labeled fertilizer (34.78 gram) using Eq. 6.2, and apply $^{15}$N-labeled urea evenly by hand to the first sub-plot. One shall be aware that $^{15}$N-labeled fertilizer such as urea come as a fine particle therefore extreme care shall be taken while applying to ensure its even application. Fine sand of the same diameter or any other inert material shall be mixed with the $^{15}$N-labelled urea to ensure even application. One shall also avoid $^{15}$N labelled urea under windy conditions or when a heavy rainfall is expected. If irrigation water is available, it is important that the experimental plots are supplied with at least 10–20 mm of irrigation soon after N fertilizer application to move urea from surface into the soil to minimize the risk of ammonia volatilization.
- When the time arrives for the 2nd split $^{15}$N fertilizer application, place a plastic sheet/cover around the perimeter of only the second sub-plot of 4 m$^2$ (this sub-plot will have previously received only ordinary urea) to ensure that ordinary urea is applied only to all areas of the main plot except the 2nd sub-plot during the 2nd fertilizer application. Then, uniformly apply the required amount (0.39 kg) of ordinary urea to the entire 45 m$^2$ of the larger main plot, but exclude the 2nd sub-plot.
- Remove the plastic sheet, and carefully apply the required amount (34.78 gram) of $^{15}$N-labeled fertilizer to the 2nd sub-plot as above.
- Carry out normal farm practices like spraying of herbicides and insecticides, and apply normal irrigation volumes until the wheat crop reaches its maturity.
- At the appropriate time, harvest the wheat crop from each sub-plot. For $^{15}$N uptake by below ground (roots) and aboveground plant parts (i.e., stems, leaves and grain), randomly select 3–4 wheat plants from the **middle row** of each sub-plot of $^{15}$N; and transfer them to plastic bags. After transporting wheat plant samples to the lab, separate the plant samples into (1) roots, (2) stem and leaves and (3) grain. Wash gently the plant tissue with tap water first, then with distilled water. After washing, allow water to drain and then dry the three types of wheat tissue samples at 65 °C for 7 days or until samples are dried to a constant weight.
- After drying, grind the wheat roots, leaves and stems and grain samples separately to a fine powder (for determination of the total N by Kjeldahl or by the **combustion** method). Then, accomplish the $^{15}$N determination by stable isotope mass

spectrometry. Be certain to clean the grinder with a brush (and also use a blower), in between grinding the individual plant tissue samples.
- Also collect four soil samples (each 0–15 cm soil depth) from each of the two sub-plots; mix them to get one composite soil sample for $^{15}$N and total N analysis.

**Wheat Straw and Grain Yield**

- To determine wheat yield, select 3 m × 3 m area within each main-plot (7 m × 7 m) and harvest wheat crop at the same time as above for $^{15}$N analysis. Then, separate the biomass into (1) shoot and leaves and (2) grain and record their fresh bulk weight immediately.

**[Note: Researchers must not use the small $^{15}$N plot for biomass production]**

- To determine moisture fraction in leaves plus stems (straw) and in grain, select 2 to 3 randomly chosen wheat plants, from each 3 m × 3 m plot; transfer them to plastic bags, seal each plastic bag using a rubber band to ensure that no water losses occur from the collected plant tissue. After transporting the wheat plant samples to the lab, separate the plant samples into straw and grain, and record their fresh weight. Wash them with tap water to remove the soil. Then take sub-tissue samples of each type of plant tissue (grain and straw), followed by drying the sub-samples of tissue at 65 °C for 7 days.
- Record the dry weights of the plant tissue after 7 days in order to calculate their moisture contents. This will provide the researcher with wheat dry matter yield (**DM**) per hectare as shown in Eq. (6.3).

$$\text{Wheat straw or grain DM (kg per ha)} = \text{FB Wt (kg)} \times \frac{10,000\ \text{m}^2}{\text{harvested area (m}^2\text{)}} \times \frac{\text{SD Wt (kg)}}{\text{SF Wt (kg)}} \tag{6.3}$$

Where, FB Wt is fresh bulk weight (kg per m$^2$) of the harvested area of the sub-plot (area (3 m × 3 m), and SD Wt and SF Wt are sub-plot sample's dry and fresh weights, respectively.

## *4.2 Calculation of Nitrogen Use Efficiency (NUE)*

The following example provides step-by-step guidance for estimating fertilizer 'N use efficiency' of a wheat crop.

A field study was carried out with a wheat crop to assess the fertilizer N use efficiency of wheat grain which received nitrogen fertilizer at the rate of 80 kg N ha$^{-1}$ in 2 split doses (40 kg N ha$^{-1}$ for each of two application times). The experimental sub-plot was 4 m$^2$ in size and the $^{15}$N fertilizer was labeled with exactly 5% atom excess. At the end of growth period, assuming the grain yield from harvested wheat was 2667 kg per ha and the N content in the grain, as obtained by Kjeldahl

analysis was 3.0%, the amount of total N removed from the soil by the wheat grain is calculated below (Eq. 6.4):

$$\text{Wheat grain N uptake (kg N per ha)} = \frac{\text{grain yield (kg per ha)} \times \text{total N (\%) of grain}}{100} \tag{6.4}$$

$$\text{wheat grain N uptake} = \frac{2667 \times 3}{100} = 80 \text{ kg N per ha}$$

The grain $^{15}N$ measurements from the 1st and 2nd split applications of $^{15}N$-labeled fertilizers showed that an 'atom excess percentage' of 0.75% and 0.80% occurred, for the two sub-plots. The fertilizer N use efficiency of the grain is calculated as follows:

(i) Percentage grain N derived from 1st and 2nd fertilizer application (*% Ndff*), based on the ratio of grain $^{15}N$ [0.75% and 0.80%, to fertilizer $^{15}N$ (5%)], can be calculated from Eq. 6.5.

$$\%Ndff = \frac{^{15}N_{grain}}{^{15}N_{Fertilizer}} \times 100 \tag{6.5}$$

*% Ndff* for the 1st application $= \frac{0.75}{5} \times 100 = 15\%$
*% Ndff* for the 2nd application $= \frac{0.80}{5} \times 100 = 16\%$
*% Ndff* for the two split applications $= 15 + 16 = 31\%$

(ii) From the *% Ndff*, the amount of N derived from the two split fertilizer applications (*Ndff*) is calculated as:

$$Ndff = \%Ndff \times \text{N taken up by crop} \tag{6.6}$$

$$Ndff = \frac{31}{100} \times 80 = 24.8 \text{ kg N per ha}$$

**[Note**: The above equations (Eqs. 6.5 and 6.6) can also be used to calculate *Ndff* of the aboveground wheat plant tissues (straw) as well as roots, if such information is needed.]

Finally, fertilizer N use efficiency *(FNUE)* is calculated from *Ndff* (24.8) and N rate applied (80 kg N $ha^{-1}$).

$$FNUE = \frac{Ndff}{\text{Total fertilizer } N_{\text{applied}}} \times 100 \tag{6.7}$$

$$FNUE = \frac{24.8}{80} \times 100 = 31\%$$

Thus, in this study the wheat grain derived **31**% of its N from the applied $^{15}N$-labeled urea fertilizer, with the remaining N (**69%**) coming from the pre-existing soil N pool.

### 4.3 An Example for $^{15}$N-Labeled Urea Dilution

For diluting 1 kg of $^{15}$N-labeled urea with 5 atom–3 atom %, please see the calculations below (Eq. 6.8) using a mixing model based on the following relationship:

$$f_A + f_B = 1 \tag{6.8}$$

Where, $f_A$ and $f_B$ refer to the fractions of labeled fertilizer and un-labeled fertilizers, respectively.

- First calculate the fraction of $^{15}$N-labeled fertilizer with 5 atom % ($f_A$) which will be required for mixing with un-labeled fertilizer to make 3 atom % using Eq. 6.9 below:

$$f_A = \frac{3 - 0.366}{5 - 0.366} = 0.56841 \tag{6.9}$$

- Then calculate the fraction of un-labeled fertilizer using Eq. (6.10) below:

$$f_B = 1 - 0.5684 = 0.43159 \tag{6.10}$$

Thus, for 1 kg of labeled fertilizer with 3 atom %, weigh 0.56841 kg of 5 atom % fertilizer and mix it with 0.43159 kg of un-labeled fertilizer.

## 5 Biological Nitrogen Fixation (BNF)

Over the past 62 years, world food supplies have become heavily dependent on the use of synthetic N fertilizers predominantly urea, with over half of this N fertilizer being applied to cereal crops. The use of fertilizer N will continue to play a critical role in ensuring world food security. Currently, world fertilizer N use is 113 million metric tons (2016), and this use is expected to increase to 120 million metric tons in 2018. Most of these increases in N fertilizer use will occur in developing countries.

Since the oil crisis of 1974 (and high N fertilizer prices), research attention of many international programs has focused on the use of biological N fixation (BNF) as an alternative N source in agro-ecosystems. Under this natural process, micro-organisms convert atmospheric N ($N_2$) into ammonia through enzymatic (nitrogenase) reactions for further utilization of the reduced N in plant metabolism. These $N_2$-fixing micro-organisms can live alone in the soil or in symbiosis with some plant species in a wide range of environments.

A classical example occurring in agricultural systems is the symbiotic association between *Rhizobium* bacteria and the roots of legumes in the Fabaceae family of plants (grain legumes, forage and pasture legumes and a number of tree species). Plant species in the Fabaceae are widely distributed in the world. In this symbiosis,

the bacteria inoculate the roots of the legumes, and form nodules which are filled with bacteroids (an altered form of the bacteria).

Legume species are common sources of protein-rich food for humans and feed for their livestock, and they also provide fiber, medicines and other products. Grain legumes can be cultivated in a separate crop rotation, or by intercropping with cereals. The forage legume species are normally used in mixed swards. The tree legume species are employed in agro-forestry and agro-sylvo-pastoral systems. Certain fast-growing legume species may be included in cropping systems for use as cover crops, or incorporation into the soil as green manures.

In order to ensure appreciable biological nitrogen fixation (BNF) inputs into agricultural production systems, legume genotypes can be grown from seeds, or propagated vegetatively. Then, selected biofertilizers (commercially available *Rhizobium* cultures) are applied as inoculants to the seeds or seedlings, or to rooted cuttings for tree species. The amount of $N_2$ fixed by the legumes depends on the symbiosis established between the *Rhizobium* strain and the legume species. Here, the cultivar (genotype) as well as environmental (soil, climate) and agronomic management factors are also important. A number of stress conditions, such as salinity, acidity, drought, extreme temperatures and nutrient deficiencies have negative effects on both partners of the symbiosis.

Appreciable amounts of $N_2$ are fixed by legumes, thereby contributing to an improved soil fertility status and reducing the need for chemical fertilizer N. A significant proportion of this fixed N is utilized by the cereal crops or grasses which are grown in association with the legumes, or in a crop rotation with the legume. Other apparent benefits called 'legume effects', are also attributed to the inclusion of the legume into the agricultural system. Table 6.2 provides a summary of the legume's effects in agro-ecosystems.

Any program aimed at enhancing the use of legume BNF for improving soil fertility and crop productivity in cropping systems should include the ability to measure $N_2$ fixation under a wide range of environmental and agronomic management conditions. Methods to assess legume $N_2$ fixation under field conditions can be grouped into isotopic and non-isotopic methodologies.

## 5.1 Estimating Legume BNF Using $^{15}N$ Isotope Techniques

Isotopic methods using the stable $^{15}N$ isotope, both with enrichment and also at natural abundance levels, provide the most sensitive measures of total $N_2$ fixation over the growing cycle of legume crops. These are also the only methods capable of distinguishing atmospheric $N_2$ from other sources of N present in the soil.

Of the two main stable isotopes of N, the light isotope $^{14}N$, is by far the most abundant (99.6337%). The heavy stable isotope $^{15}N$, has an abundance of 0.3663 atom %. If the $^{15}N$ concentrations within each of the two main sources of N (atmospheric $N_2$ and soil N) differ appreciably, then it is possible to calculate the

**Table 6.2** Main effects of legumes in agro-ecosystem

| Issues/processes | Main effect | Details |
|---|---|---|
| BNF process *per se* | Soil acidification | Increase in $CO_2$ fixed/$N_2$ assimilated |
| | | Soil N uptake also increased |
| N fertilizer production and application | Reduction in fertilizer N use | Fossil fuel energy use reduced |
| | | $CO_2$ emissions reduced |
| | | $NO_2$ emissions reduced |
| N cycling/N losses | Effects occur during both pre-cropping and cropping | $N_2O$ emissions reduced |
| Cropping systems | | Volatilization as $NH_3$ reduced |
| | | N leaching reduced |
| | | Usually the NUE of N derived from green manure is lower than N-fertilizer, but large fraction of N-green manure remain in the soil. |
| | Post-harvest effects | Reduced $N_2O$ emissions, |
| | | $NH_3$ volatilization, and $NO_3^-$ leaching |
| | | N benefits to next crop/savings from not having to apply as much fertilizer N |
| | Long-term effects | Soil fertility improvement |
| | | Soil N reserves increased |
| | | Risk of N losses reduced for intensive cropping systems |
| Use of legume crops | Non-N 'Legume' effects also promoted | Human health improved (quality food diet) |
| | | Biodiversity increased |
| | | Carbon sequestration enhanced |
| | | Soil erosion reduced |
| | | Can interrupt crop pest and disease cycles |
| | | Deep rooting promoted |
| | | Soil structure improved |

proportion of the total N that accumulates within the legume tissues that is derived from atmospheric $N_2$ fixation.

When the aim is the assessment of the N input by $N_2$-fixing plants through BNF, three parameters are required: the content of N in plant material, the dry matter yield of the $N_2$-fixing plant and the percentage of N in the $N_2$-fixing plant derived from the atmosphere (%Ndfa). Considering these three parameters, it is possible to calculate the amount of N fixed, usually expressed in terms of kg N derived from BNF per ha, in field experiments, or mg N derived from BNF per plant or per pot in glasshouse experiments. Based on these estimates, it is also possible to calculate the amount of N derived from soil by discounting the amount of N derived from BNF from the total N.

The %Ndfa depends on the interaction between plant growth and efficiency of microsymbiont strain. It is also depends on the soil physical and chemical properties, (e.g., water and nutrient availability). The two most important isotopic techniques for this purpose are the $^{15}N$ isotope dilution and $^{15}N$ natural abundance technique (Boddey et al. 2000; Urquiaga et al. 2012; Collino et al. 2015). Other isotopic

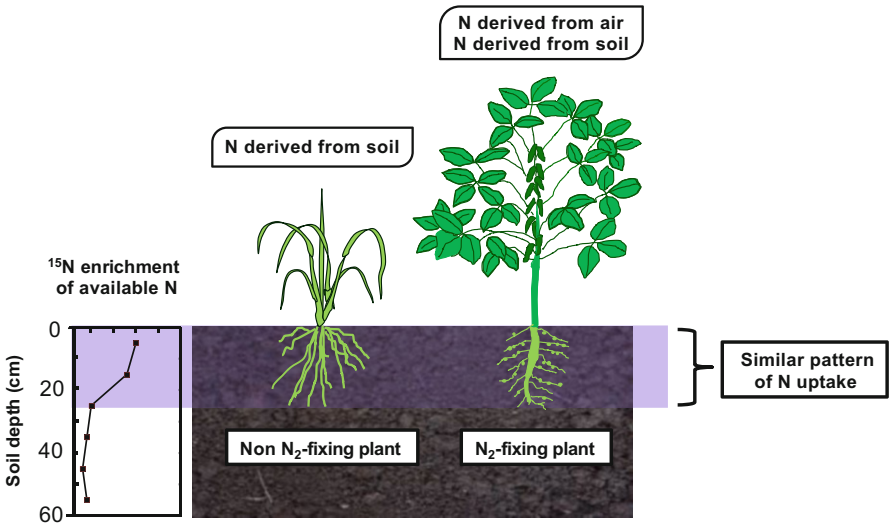


**Fig. 6.4** Illustration of the $^{15}N$ isotope dilution technique for the BNF quantification

techniques, such as $^{15}N_2$ feeding and A-value can be also applied depending on the purpose of the BNF quantification, for which detailed procedures can be found in previous literature (*e.g.*, IAEA 2001).

## 5.2 *$^{15}N$ Isotope Dilution Technique*

The $^{15}N$ isotope dilution technique has been the most applied isotopic technique for %Ndfa assessment. This technique is based on the dilution of soil N taken up by the $N_2$-fixing plant by N derived from air through BNF (Fig. 6.4). When this technique is applied it is assumed that the $^{15}N$ enrichment of non $N_2$-fixing plant can be used as reference to assess the $^{15}N$ enrichment of plant-available soil N (Fig. 6.4).

To apply this technique, the soil N taken up by plants is labelled through application of $^{15}N$-enriched fertilizers. After the labelling, both $N_2$-fixing and non $N_2$-fixing reference plants are grown and sampled at the same time. In fact, if all N forms in soil were easily mineralisable and available for plant uptake, the direct $^{15}N$ analysis of soil samples could be used as reference to assess $^{15}N$ abundance of N fraction in $N_2$-fixing plants derived from soil. However, only the soil mineral N forms (mainly $NH_4^+$ and $NO_3^-$), representing a small fraction of N, is available for plant uptake and could theoretically be used to assess the $^{15}N$ abundance of the N in plants derived from soil (Ledgard et al. 1984; Unkovich et al. 2008). Considering that non $N_2$-fixing plants has their N nutrition totally dependent on soil mineral N, these plants can be sampled to assess $^{15}N$ enrichment of the plant-available soil N (Fig. 6.4). In this technique, $N_2$-fixing plant and the non $N_2$-fixing plant (reference)

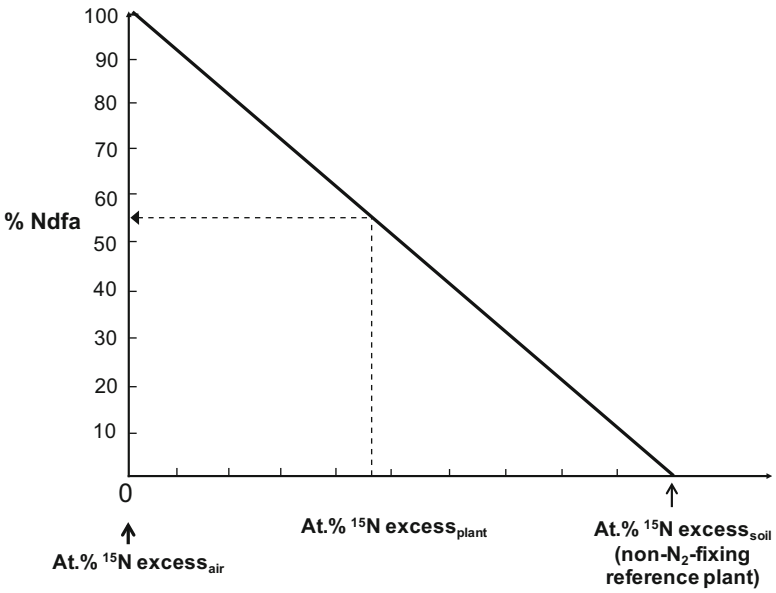


**Fig. 6.5** Relationship between $^{15}N$ enrichment of $N_2$-fixing plant (abscissa axis) and percentage of N derived from atmosphere (%Ndfa, ordinate axis)

should have similar pattern of N uptake (Fig. 6.4). This is a critical prerequisite for application of $^{15}N$ isotope dilution technique because, otherwise, the assessment of %Ndfa can be inaccurate when $^{15}N$ enrichment of soil N is not constant in the time course and/or in the depths of soil N uptake by fixing and non-fixing plants (Baptista et al. 2014; Unkovich et al. 2008). Some procedures can be useful to deal with the non-constant $^{15}N$ enrichment in time and soil depth, including the use of labile organic materials to immobilise excessive soil mineral N and stabilize N supply over time (Boddey et al. 1995) and constant addition of $^{15}N$-labelled fertiliser to the soil (Viera-Vargas et al. 1995). The %Ndfa by $N_2$-fixing plants is calculated using the following Eq. 6.11:

$$\%\text{Ndfa} = 1 - \frac{\text{atom}\%^{15}\text{N excess}_{\text{N}_2\text{fixing plant}}}{\text{atom}\%^{15}\text{N excess}_{\text{non N}_2-\text{fixing reference plant}}} \times 100 \qquad (6.11)$$

The graphical representation of Eq. 6.11 is showed in Fig. 6.5. Taking in consideration that N fertiliser rate can impact the BNF process, it is usual to apply low N rates (e.g., <10 kg N $ha^{-1}$) when the objective is solely the labelling of plant-available soil N with $^{15}N$. When using low rates of N, fertiliser with high $^{15}N$ enrichment is usually applied to yield plant materials with $^{15}N/^{14}N$ ratios adequate for precise and accurate analyses by spectrometry. The application of 1 kg of $^{15}N$ excess per hectare (0.1 g $^{15}N$ excess $m^{-2}$) usually yields plant materials with sufficient $^{15}N$ enrichment to be analysed with acceptable precision by most of

mass spectrometers (emission spectrometers commonly requires higher $^{15}N$ enrichments). Considering these values, if a rate of 10 kg N $ha^{-1}$ should be applied, the use of a fertiliser with 10 atom% $^{15}N$ excess would be recommended. In fact, there is a possibility of using lower $^{15}N$ enrichments depending on the spectrometer type, but this must be based on a rigorous assessment of analytical precision and after significant experience was gained. When this methodology is used for woody perennials, higher N rates (e.g., 20 kg N $ha^{-1}$) and/or $^{15}N$ enrichments should be used.

The selection of non $N_2$-fixing plants is a very important step for the accurate quantification of BNF by $^{15}N$ isotope dilution technique. Some recommendations are presented below to avoid some biases due the selection of non $N_2$-fixing reference plants:

- To be sure that the reference plants do not have the ability of $N_2$-fixing, which could be identified by:

  Classical N deficiency symptoms (*e.g.*, pale green or yellow colour, especially in the older leaves).

  Literature search indicating the inability of $N_2$-fixing. That is especially important for Poaceae, considering that some species of this plant family has the ability of $N_2$-fixing (Urquiaga et al. 1992; Reis et al. 2001).

  Absence of nodules when non-nodulating isolines or non-inoculated legumes are used as reference plants.

- To use three or more reference plant species to assess the variability associated with the $^{15}N$ enrichment of plant available soil N.
- Select non reference plants that presents patterns of N uptake similar to that of $N_2$-fixing plant, that is, have similar rooting depth and architecture exploiting the same pool of plant-available soil N and have the same dynamics of N uptake over time;
- If different varieties of a $N_2$-fixing crop having significant different life cycles are to be compared for the BNF ability, the group of varieties with similar life cycle must be paired with a reference plants with the duration of growth.
- Considering that differences in soil history can affect N mineralisation dynamics, additional reference plants must be grown and sampled for each different crop sequence even when BNF will be assessed for only one $N_2$-fixing crop type (e.g., effect of cropping history on BNF associated to soybean);
- Ideally, each reference plant should be considered as an additional treatment in the layout of field and glasshouse experiments, that is, they should be grown in additional field plots with the same replication and randomisation made for $N_2$-fixing crops.

To apply $^{15}N$-fertilisers aiming to label the plant-available soil N, the same strategy of $^{15}N$-microplot inside the main field plot previously described to study Fertilizer Use Efficiency can be used for BNF quantification using $^{15}N$ isotope dilution technique. The plant material sampled in micro-plots will provide an estimate of %Ndfa. The dry matter yield, the total N taken up and the amount of N

derived from BNF (e.g., kg N-BNF $ha^{-1}$) can be measured by harvesting larger area of the plot, including the area that received $^{14}N$-fertiliser.

## 5.3 Calculation of the Amount of N Derived from BNF by $^{15}N$ Isotope Dilution Technique

The following example shows the steps for estimating the %Ndfa the amount of N derived from BNF, in kg N $ha^{-1}$, for soybean crop by $^{15}N$ isotope dilution technique. A field study was carried out with a commercial soybean variety to assess the performance of three *Rhizobium* strains under a condition of water stress. The soybean was sown at row spacing of 0.50 m and three plant species were included as non $N_2$-fixing reference plants: *Sorghum* sp.; *Brassica* sp. and non-nodulating soybean. The quantification of BNF will be performed by $^{15}N$ isotope dilution technique. Each experimental plot was 36 $m^2$ (6 m × 6 m). A micro-plot was established in an area of 9.0 $m^2$ (3.0 m × 3.0 m) in each experimental plot. For this study, $^{15}N$-labeled ammonium sulphate ($(^{15}NH_4)_2SO_4$) with enrichments of 20 atom % $^{15}N$ in excess was applied 50 days before sowing to each micro-plot at a rate of 5 kg N $ha^{-1}$. Non-labelled fertiliser ($(^{14}NH_4)_2SO_4$) was also applied the remaining area of the plot. The soybean and reference plants were sown and harvested (105 days after sowing) concomitantly. The plants (shoot tissue) corresponding 1.5 m of the central row of the $^{15}N$-labelled micro-plot were collected, weighted, oven-dried, reweighted, ground and analysed for total N and $^{15}N$. Dry mass, N content and $^{15}N$-erichment are presented below (Table 6.3).

The mean value of $^{15}N$ enrichment of reference plants was 1.1305 atom % $^{15}N$ excess. An example calculation for the soybean inoculated with strain A is presented as follows using Eqs. 6.12 and 6.13:

$$\text{Total N in shoot (kg per ha)} = \frac{\text{Dry mass (kg per ha)} \times \text{N content (\%)}}{100} \quad (6.12)$$

**Table 6.3** Example of results of field experiment with soybean for quantification of BNF by $^{15}N$ isotope dilution technique

| Parameter | Soybean inoculated with strain A | Soybean inoculated with strain B | Soybean inoculated with strain C |
|---|---|---|---|
| Dry mass (kg $ha^{-1}$) | 5097 | 4850 | 3105 |
| N content (%) | 3.7 | 3.9 | 3.7 |
| Atom % $^{15}N$ excess | 0.1420 | 0.0330 | 0.0920 |

$$\text{Total N in shoot} = \frac{5097 \times 3.7}{100} = 189 \text{ kg N per ha}$$

$$\%\text{Ndfa} = 1 - \frac{\text{atom}\%^{15}\text{N excess}_{\text{N}_2\text{fixing plant}}}{\text{atom}\%^{15}\text{N excess}_{\text{non N}_2-\text{fixing reference plant}}} \times 100$$

$$\%\text{Ndfa} = 1 - \frac{0.1420}{1.1305} \times 100 = 87\%$$

$$\text{Amount of N derived from BNF (kg per ha)} = \frac{\text{Total N in shoot (kg per ha)} \times \%\text{Ndfa}}{100} \tag{6.13}$$

$$\text{Amount of N derived from BNF} = \frac{189 \times 87}{100} = 165 \text{ kg N per ha}$$

Considering the other data of shoot dry mass, N content and atom % $^{15}$N excess, the amounts of N derived from BNF for soybean inoculated with strain B was 184 kg N ha$^{-1}$ and for soybean inoculated with strain C was 106 kg N ha$^{-1}$.

## 5.4 *$^{15}$N Natural Abundance Technique*

This technique depends on the slight natural enrichment of $^{15}$N in the soil, relative to atmospheric $N_2$. The slight increase of $^{15}$N in soil is a consequence of the non-identical behaviour of the light and heavy isotopes involved in various reactions in the soil environment. The $^{15}$N isotopic fractionation, also called the mass discriminatory effect (Xing et al. 1997), is a result of complex and prolonged interaction of biological, chemical and physical processes in soils, which results in fractionation between $^{15}$N and $^{14}$N. There is a tendency of the reaction products, such as the gaseous N forms produced by denitrification, to become relatively enriched in the lighter isotope $^{14}$N, while the remaining N compounds, which can be stabilised in soil organic matter over time, tend to be enriched in the heavier isotope $^{15}$N (Xing et al. 1997). It is important to consider that this small $^{15}$N enrichment occurs in a long time scale, and is closely associated to soil organic matter retention and long-term dynamics (Ledgard et al. 1984).

Considering that $^{15}$N natural abundance technique is based on the analyses of plant samples having very small $^{15}$N deviation relative to atmospheric $N_2$, it is usual to express the results of $^{15}$N natural abundance analyses in terms of δ units. The $\delta^{15}$N value is the difference in the ratio $^{15}$N:$^{14}$N of a given sample and the ratio $^{15}$N:$^{14}$N in the nominated international standard of atmospheric $N_2$, expressed by parts per thousand (‰). One unit of $\delta^{15}$N (1.0‰) is a thousandth of the $^{15}$N natural abundance of the atmosphere (0.3663 atom% $^{15}$N) above or below the natural abundance of atmospheric $N_2$, that is, one unit of $\delta^{15}$N it is equal to 0.0003663 atom% $^{15}$N excess. The following Eq. 6.14 is applied to calculate the $\delta^{15}$N:

$$\delta^{15}\mathrm{N}(‰) = \frac{\mathrm{atom}\%^{15}\mathrm{N}_{\mathrm{sample}} - \mathrm{atom}\%^{15}\mathrm{N}_{\mathrm{atmosphere}}}{\mathrm{atom}\%^{15}\mathrm{N}_{\mathrm{atmosphere}}} \times 1000 \quad (6.14)$$

Therefore, the $\delta^{15}N$ of atmospheric $N_2$ will be by definition equal to 0‰. Positive value of $\delta^{15}N$ means that there are an enrichment of $^{15}N$ in the sample compared to the atmospheric $N_2$ and negative values means that the sample presents a slightly depletion. For example, if a plant sample has 0.35855 atom% $^{15}N$, the resulting $\delta^{15}N$ of this sample is:

$$\delta^{15}\mathrm{N} = \frac{0.3659 - 0.3663}{0.3663} \times 1000 = -1.09‰$$

The main advantage of the natural abundance technique, compared to $^{15}N$ isotope dilution technique, is the no requirement to add $^{15}N$ fertiliser to label the soil available N, which is a very expensive consumable and, depending on the N rates, it can affect BNF process. However, an important disadvantage of this technique is the need for an Isotope Ratio Mass Spectrometer with high precision.

The Eq. 6.15 can be used to calculate the Ndfa% by using the $^{15}N$ natural abundance technique is:

$$\mathrm{Ndfa}\% = \frac{\delta\,^{15}\mathrm{N}_{\text{reference plant}} - \delta\,^{15}\mathrm{N}_{\text{fixing plant}}}{\delta\,^{15}\mathrm{N}_{\text{reference plant}} - B} \times 100 \quad (6.15)$$

where B is the $\delta^{15}N$ for the $N_2$ fixing plant when completely dependent on $N_2$ fixation for growth. The B value is usually negative as a result of isotopic fractionation within the legume. The value of B depends on the plant species, plant age, symbiont and growth conditions. Unkovich et al. (2008) presented some tables with compilation of a wide number of B values for shoot of many tropical and temperate legumes, which can be used to estimate %Ndfa with an acceptable accuracy depending on the $N_2$ fixation level.

Another important factor affecting the %Ndfa estimate is the $\delta^{15}N$ of the reference plant. The higher is this parameter, the better is the estimate of %Ndfa because this will result in less impact of biases associated to small variability of some processes, such as the mineralisation intensity of soil N pools, isotopic discrimination in plants or small differences in root architecture between $N_2$-fixing and non-fixing reference plants. Reference $\delta^{15}N$ higher than 4‰ have been considered suitable for estimating %Ndfa in $N_2$-fixing plants (Unkovich et al. 2008).

An important practical procedure to have an initial estimate of $^{15}N$ natural abundance of plant-available soil N before the beginning of the experiment is the $^{15}N$ analysis of non $N_2$-fixing broadleaf and grass weeds in the experimental area available for BNF studies. Separated samples of the different reference plant should be collected in different points of the area to assess the variability of $\delta^{15}N$ in plant-available N (not a composite sample). In addition to that, details on the history of the area are very useful, including previous crop type, N fertilisation (type and rates) and use of inoculants.

**Table 6.4** Example of results of glasshouse experiment for measuring BNF associated to *Phaseolus vulgaris* by $^{15}N$ natural abundance technique

| Parameter | Common bean variety A | Common bean variety B |
|---|---|---|
| Dry mass (g per pot) | 45 | 39 |
| N content (%) | 2.5 | 2.6 |
| $\delta^{15}N$ | 0.52 | 0.96 |

All recommendation presented for $^{15}N$ isotope dilution technique to select non $N_2$-fixing plants must also be considered for the $^{15}N$ natural abundance technique. The reference plants must be considered as additional treatments in the experimental design, with replication and randomisation. When experiments are conducted as randomised block design the %Ndfa estimate for the plants of a given block should be performed with the $\delta^{15}N$ of the references of the same block individually.

**Calculation of the Amount of N Derived from BNF by $^{15}N$ Natural Abundance Technique** The following example shows the steps for estimating the %Ndfa and the amount of N derived from BNF, in kg N $ha^{-1}$, for common bean (*Phaseolus vulgaris, L.*) by $^{15}N$ natural abundance technique:

A glasshouse study was carried out with two varieties of common bean to assess the osmotic effect of a salt (NaCl) on the BNF performance. The common bean cultivars and three reference plants (*Sorghum* sp.; *Brassica* sp. and non-nodulating bean) were sown in 10-L pots with 10 kg of soil. Three plants were used per each pot. Soil salinity was simulated by adding NaCl solution in soil. The BNF quantification will be performed by $^{15}N$ natural abundance technique. The common bean shoots were collected at 60 days after sowing, weighted, oven-dried, reweighted, ground and analysed for total N and $^{15}N$. Dry mass, N content and $^{15}N$ abundance are presented below (Table 6.4).

The mean value of $\delta^{15}N$ of reference plants was 9.82‰ and the B value used for common bean was −1.97‰. An example calculation for the variety A is presented as follows:

$$\text{Total N in shoot (g per pot)} = \frac{\text{Dry mass (g per pot)} \times \text{N content (\%)}}{100}$$

$$\text{Total N in shoot} = \frac{45 \times 2.5}{100} = 1.13 \text{ g N per pot}$$

$$\text{Ndfa\%} = \frac{\delta\,^{15}\text{N}_{\text{reference plant}} - \delta\,^{15}\text{N}_{\text{fixing plant}}}{\delta\,^{15}\text{N}_{\text{reference plant}} - B} \times 100$$

$$\text{Ndfa\%} = \frac{9.82 - 0.52}{9.82 - (-1.97)} \times 100 = 79\%$$

$$\text{Amount of N derived from BNF (mg per pot)} = \frac{\text{Total N in shoot (g per pot)} \times \%\text{Ndfa}}{100}$$

$$\text{Amount of N derived from BNF} = \frac{1.13 \times 79}{100} \times 1000 = 893 \text{ mg N per pot}$$

Considering the other data of shoot dry mass, N content and atom % $^{15}$N excess, the amounts of N derived from BNF for variety B was 761 mg N per pot.

## 5.5 Correction for N Derived from Seed

In some experiments using plants with proportionally large seeds or when plants are sampled in early growth stages, when N derived from seeds can supply a significant proportion of plant N, a correction in $^{15}$N enrichment/abundance of plant materials can improves the accuracy of the %Ndfa estimate (Okito et al. 2004). This correction is made by subtracting the amounts of N derived from seed and its $^{15}$N enrichment/abundance from plant material. For example, the following Eq. 6.16 is applied for correction when $^{15}$N natural abundance is applied:

$$\delta^{15}N_{\text{plant (SC)}} = \frac{\left(\%N_{\text{plant}} \times DM_{\text{plant}} \times \delta^{15}N_{\text{plant}}\right) - \left(\%N_{\text{seed}} \times DM_{\text{seed}} \times P_s \times \delta^{15}N_{\text{seed}}\right)}{\left(\%N_{\text{plant}} \times DM_{\text{plant}}\right) - \left(\%N_{\text{seed}} \times DM_{\text{seed}}\right)} \tag{6.16}$$

where SC indicates the correction for seed N, %N is the N content, DM is the dry mass, $P_s$ is the proportion of the seed N assimilated by plant tissue. $P_s$ is usually assumed to be 0.5 when shoot tissue is analysed considering that half of N seed is incorporated in into the aerial tissue. The same equation can be applied for $^{15}$N isotope dilution technique by replacing the values of $\delta^{15}$N by atom% $\delta^{15}$N excess. When plants grown under field conditions and are sampled at the maturity stage this correction does not usually have a significant influence in the final estimate of % Ndfa because the contribution of seed N in this case is commonly small.

**General Comments:**
The use of $^{15}$N techniques has been successfully applied to measure BNF in many agricultural systems in many regions of the world. However, before the beginning of the experimentation using those isotope techniques it is important to take into account the main requirements needed for success in the BNF measurement: (i) the requirement of highly skilled workers for all activities from the selection of the experimental area to the interpretation of the $^{15}$N analysis, and (ii) the requirement of financial resources considering that consumables for $^{15}$N analysis are usually expensive compared to other routine plant and soil analyses. The selection of the most appropriate technique will depend mainly on the precision of the Mass-

**Table 6.5** Some advantages (A) and disadvantages (D) of two $^{15}N$ isotope techniques for measuring BNF in agricultural systems

| Criteria | $^{15}N$ isotope dilution technique | $^{15}N$ natural abundance technique |
|---|---|---|
| Requirement of reference plants | D | D |
| Cost with $^{15}N$ fertiliser | D | A |
| Cost with $^{15}N$ analysis | D | D |
| Requirement of high-skilled technicians | D | D |
| Requirement of high-precision spectrometers | A | D |
| Application of the technique in areas (with grown plants) not initially designed for BNF assessment (*e.g.*, farms, natural systems) | D | A |
| Need of considering isotope fractionation (B value) | A | D |
| Field variability of soil $^{15}N$ | A | D |
| Application in perennial systems | A | A |
| Application in experiments with soils presenting plant-available N with low $\delta^{15}N$ (<4‰) | A | D |
| Time integrated measurement of %Ndfa | A | A |
| Measurement of amount of N derived from BNF per area (field) or per pot (glasshouse) | A | A |

Spectrometer used for $^{15}N$ analysis of plant materials. Other criteria are also presented in Table 6.5.

## 6 Water Stable Isotope Technique to Determine Evapotranspiration Partitioning

In agriculture, evapotranspiration (ET), or the flux of water from a vegetated surface via both evaporation (E) and transpiration (T) by plants, is an important component of the water budget. Water loss via transpiration can be considered 'good' water use, while water loss via evaporation can be considered 'wasted' water use (Fig. 6.6). Transpiration occurs through stomatal pores, the pores which are also used by the plants for uptake of atmospheric $CO_2$ in photosynthesis, and subsequent biosynthesis of carbon compounds, a process which ultimately leads to biomass gain. Stomata are tightly controlled by plant physiological signals to optimize carbon gain per unit of water lost. The use of the stable isotopes $^{18}O$ and $^{2}H$ as signatures in water and water vapor can help scientists to differentiate between water losses through direct soil evaporation versus transpiration from the plant leaves. That knowledge can be used to apply appropriate soil and water conservation strategies such as minimum tillage, mulching and a drip/spray irrigation system in order to minimize soil evaporation under a range of different management practices. Water use efficiency

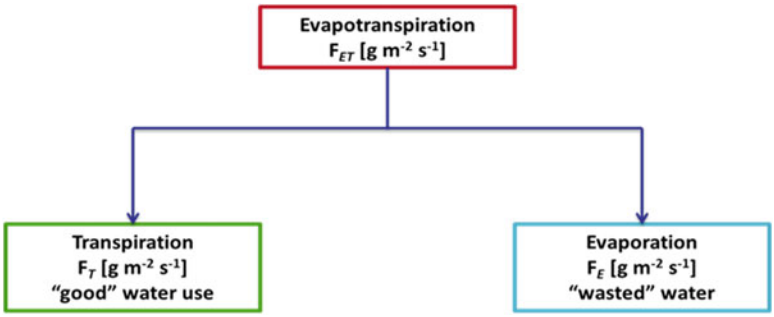


**Fig. 6.6** Evapotranspiration model

(WUE) of a plant species or crop type is related both to the plant's genetics, as well as acclimation by the plant to the irrigation regime.

Historically, the characterization of the plant processes involved in transpiration was performed through cumbersome and inaccurate water flux measurements. However, with the recent advancement of laser-based water vapor isotope analyzers, various calculation models have been developed to correlate the real-time, spatial, and temporal isotopic measurements with evaporation and transpiration fluxes ($F_{ET}$ and $F_T$).

According to Yakir and Sternberg (2000), the ratio of these fluxes is calculated using Eq. 6.17:

$$f_{T/ET} = \frac{F_T}{F_{ET}} = \frac{\delta_{ET} - \delta_E}{\delta_T - \delta_E} \tag{6.17}$$

Where, $\delta_{ET}$ is the isotopic composition of bulk evapotranspiration, $\delta_E$ is the isotopic composition of evaporated soil-water, and $\delta_T$ is the isotopic composition of water transpired by the plant.

In this section, we demonstrate how laser-based absorption spectroscopy, and in particular, Cavity Ring-Down Spectroscopy (CRDS), can be applied to many steps of ET analyses, including: (i) characterization of partial pressure and the isotopic composition of the vertical water vapor profiles to determine the bulk ET signal through a Keeling mixing model, (ii) the use of soil water isotopic composition, in combination with the Craig-Gordon model, to determine the evaporation flux signature, and (iii) direct measurement of the isotopic signature of transpiration occurring in leaf chambers in order to determine the isotope signature of the water source.

## *6.1 Determining $\delta_{ET}$ Using the Keeling Mixing Model*

### 6.1.1 Theory

The isotopic composition of an evapotranspiration flux can be determined by using the Keeling mixing model (1958), a model which correlates water concentration

(C) and the isotopic composition (δ) of the mixed air above the surface (A), the background air (B), and the evapotranspiration flux (ET) Eq. 6.18.

**Keeling Mixing Model**

$$C_A.\delta_A = C_B.\delta_B + C_{ET}.\delta_{ET} \tag{6.18}$$

Assuming the concentration and the isotopic composition of the background air ($C_B$, $\delta_B$) and evapotranspiration ($C_{ET}$, $\delta_{ET}$) are constant over a short period of time, Eq. (6.18) can be rearranged so that $\delta_A$ is a function of $1/C_A$. In this case, (Eq. 6.19) the intercept of a plot of $1/C_A$ (x-axis) versus $\delta_A$ (y-axis) will yield $\delta_{ET}$.

$$\delta_A = (\delta_B - \delta_{ET})\frac{C_B}{C_A} + \delta_{ET} \tag{6.19}$$

### 6.1.2 Experimental Approach

Experimentally, one can measure the isotopic composition of the mixed air, $\delta_A$, at various concentrations, $C_A$, by sampling the air at different elevations above the surface. The vertical profile provides the water concentration gradient which is required in order to determine $\delta_{ET}$.

The procedure involves the following steps.

- Sample air above the soil surface at different heights. The heights at which you sample will depend on the specifics of the ecosystem being studied.
- Connect the sample lines to a manifold using a rotary valve selector.
- If possible, use a rotary valve which can be controlled via a Picarro water isotope analyzer. For example, the Picarro L2130-*i* or L2140-*i*, can be used to select the sample line through which air will be sent to the analyzer.
- Run the analyzer in dual mode: vapor and liquid measurement allows the analyzer to self-calibrate using a liquid water standard, while the vapor mode analyzes the sampled water vapor, thereby providing isotopic composition and concentration.
- Using the analyzer's Dual Mode Coordinator, set the system to measure vapor from each sample port for 10 min (i.e., a total of 50 min for one cycle – please note 5 sampling heights in this example, Fig. 6.7). Measurements should be made at a frequency of 1 Hz.
- It is recommended that the analyzer be calibrated with liquid water standards of a known isotopic composition once every 8 h. The auto-sampler injects the liquid standard sample into the vaporizer. Each injection measurement takes 9 min and a minimum of 6 injections for each liquid standard is required.

After the analyzer measurement, results are collected and processed (averaging and normalizing for each calibration), $\delta_A$ and $1/C_A$ are plotted on a graph as shown in the Fig. 6.8. Note that $\delta_{ET}$ is the y-intercept of the regression line between $\delta_A$ and $1/C_A$.

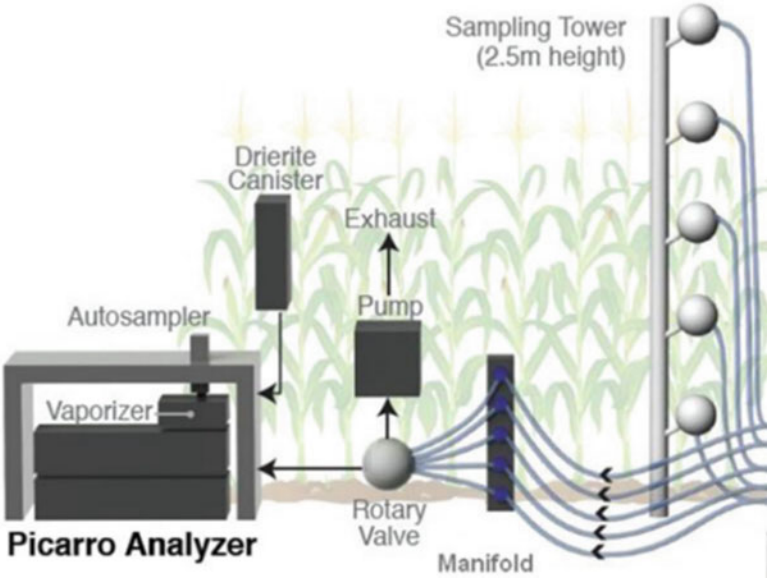


**Fig. 6.7** Example of experimental setup for sampling water vapor at different heights

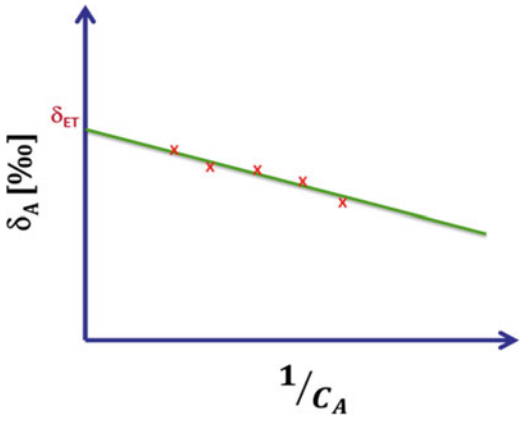


**Fig. 6.8** Example of a Keeling plot derived from a vertical profile of 5 water vapor measurements

## 6.2 *Determining $\delta_{ET}$ Using the Craig-Gordon Model*

### 6.2.1 Theory

The Craig-Gordon model (1965) is used to estimate the isotopic composition of soil-water evaporation. The model takes into account the effect of equilibrium and kinetic fractionations during the phase change between liquid to vapor (Eq. 6.20).

$$\delta_E = \frac{(\delta_L \alpha_e h_s - h'_A \delta_A) - (h_s - h_s \alpha_e) - (\varepsilon_k)}{(h_s - h'_A) + \varepsilon_k} \tag{6.20}$$

Where, $\alpha_e$ is the equilibrium vapor-liquid fractionation factor. It can be calculated as a function of soil temperature, $T_s$, [K] as explained by Majoube (1971) (Eq. 6.21).

***For $^2H$***

$$\ln \alpha_e = -52.612.10^{-3} + \frac{76.248}{T_s} - \frac{24.844.10^3}{T_s^2} \tag{6.21}$$

***For $^{18}O$*** *(Eq. 6.22)*

$$\ln \alpha_e = 2.0667.10^{-3} + \frac{0.4156}{T_s} - \frac{1.137.10^3}{T_s^2} \tag{6.22}$$

Where,

- $\delta_L$ is the soil liquid water isotopic composition [‰]
- $\delta_A$ is the ambient air water vapor isotopic composition [‰]
- $h_s$ is the soil vapor saturation which is defined by Mathieu and Bariac (1996) (Eq. 6.23):

$$h_s = e^{M\varphi_s / RT_s} \tag{6.23}$$

- $M$ is the molecular weight of water (18.0148 g/mol)
- $\varphi_s$ is the soil potential (matric potential) of the evaporating surface [kPa]
- $R$ is the ideal gas constant (8.3145 mL MPa/mol/K)
- $T_s$ is the soil temperature, i.e. the temperature of the evaporating surface [K]
- $\varepsilon_k$ is the kinetic isotopic fractionation factor (Eq. 6.24)

$$\varepsilon_k = n\left(h_s - h'_A\right)\left(1 - \frac{D_i}{D}\right) \tag{6.24}$$

- $D_i/D$, the ratio of molecular diffusion coefficients of water vapor in dry air, is taken as 0.9757 from Merlivat (1978) (Eq. 6.25):

$$h'_A = \frac{h_A e_{sA}}{a_w e_{s0}} \tag{6.25}$$

- $h'_A$ is the humidity of the atmosphere normalized to the evaporating surface
- $h_A$ is the humidity of the atmosphere
- $e_{sA}$ and $e_{s0}$ are the saturation vapor pressures at the atmosphere's (air's) temperature and the temperature of the evaporation surface, respectively
- $a_w$ is the thermodynamic activity of water

- $n$ is related to the volumetric soil moisture ($\theta_s$), the moisture of the residual ($\theta_{res}$) and the saturated moisture ($\theta_{sat}$), as proposed by Mathieu and Bariac (1996) (Eq. 6.26):

$$n = 1 - \frac{1}{2}\left(\frac{\theta_S - \theta_{res}}{\theta_{sat} - \theta_{res}}\right) \tag{6.26}$$

### 6.2.2 Experimental Approach

**Measuring $\delta_L$**
The isotopic composition of soil water will be measured using a Picarro water isotope analyzer (Fig. 6.9).

Several water extraction methods are available, as below:

**Cryogenic Distillation**
Cryogenic distillation is an established technique for extracting liquid water from samples, for example soils and leaves. Once extracted, the liquid water can be analyzed for its isotopic composition using a High Precision Vaporizer and Picarro water isotope analyzer.

**Picarro Induction Module (IM)**
The Picarro IM extracts water from soil samples by inductively heating the sample and directly sending the evaporated water vapor to the Cavity Ring-Down Spectroscopy (CRDS) analyzer. Prior to analysis on the CRDS, the water vapor is passed through a micro combustion cartridge to remove organic molecules which could potentially interfere with CRDS analysis. For more information about Picarro's Induction Module, please visit:

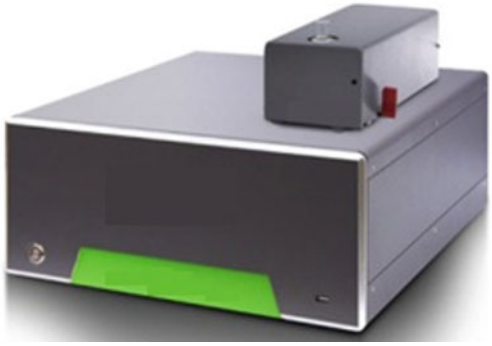

**Fig. 6.9** Induction Module and Isotopic Water Analyzer

http://www.picarro.com/isotope_analyzers/im_crds.

When extracting water from soils using either of the above methods, caution should be applied to ensure that water extraction is complete. If water extraction is not complete, it is possible that fractionation may occur during isotopic analysis (or during the extraction process), which could then lead to inaccurate results. Care should also be taken during the storage of soil samples.

**Measuring $\delta_A$ and $C_A$ and Determining $h_A$**

The isotopic composition of water vapor in the background ambient air is measured with the CRDS water analyzer when it is in the vapor mode.

Sample the ambient air well away from the studied system to ensure that no 'local' water vapor contamination occurs from evapotranspiration of the experimental plot, thereby affecting the ambient air measurement. This can be accomplished by placing the CRDS analyzer input port at an appreciable distance away from the experimental plot, or by connecting tubing to the inlet port of the CRDS in order to collect the air from well-above the canopy. The specific height above the canopy will be dependent on the ecosystem being studied.

Ensure that the CRDS analyzer is calibrated for isotopic composition and also the concentration dependence of the isotopic composition. For information on how to calibrate a Picarro Water Isotope Analyzer refer to the User's Manual. A recent version is available at:

https://picarro.box.com/s/0nh2wvm4n4ojf8jlmj7v.

The CRDS analyzer should be operated in dual mode: vapor and liquid. The liquid measurement allows the analyzer to calibrate itself with liquid standards. The vapor mode analyzes the sampled water vapor in ambient air to provide isotopic composition $\delta_A$ and concentration $C_A$.

Calculate $h_A$ using $C_A$.

## 6.3 *Determining $\delta_T$ via Direct Measurement at the Leaf*

### 6.3.1 Theory

When re-arranging the mass balance established in Eq. 6.18, we get (Wang et al. 2012):

$$\delta_T = \frac{C_M\delta_M - C_A\delta_A}{C_M - C_A} \tag{6.27}$$

Where, $\delta_A$ and $C_A$ are the isotopic composition and water concentration of the ambient air; $\delta_M$ and $C_M$ are the isotopic composition and water concentration measured from the leaf chamber, i.e. where transpiration water vapor mixes with ambient air.

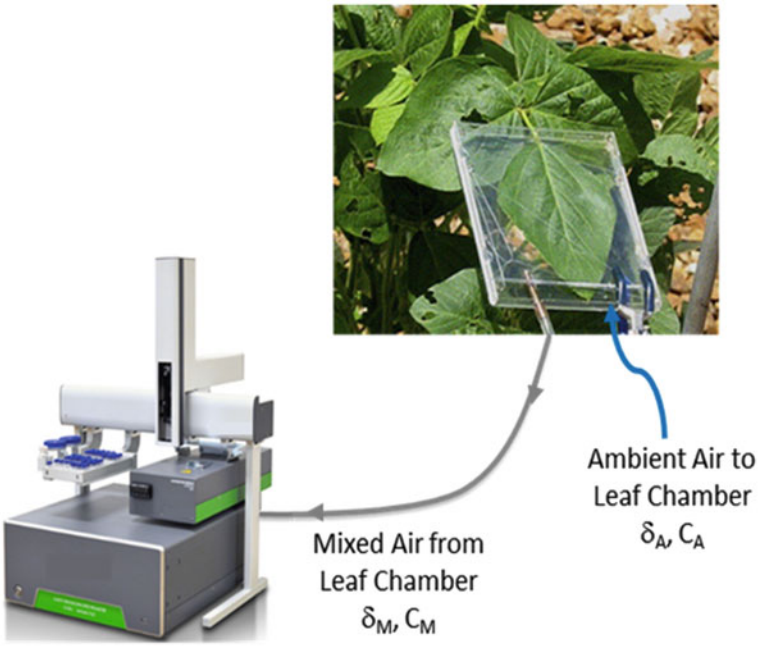


**Fig. 6.10** Experimental setup for measuring $\delta_M$ and $C_M$

### 6.3.2 Experimental Approach

**Measuring $\delta_A$ and $C_A$**

Follow exactly the procedure described on the previous page. One can directly measure the isotopic composition of the mixed air, $\delta_M$ and water concentration, $C_M$, inside of the leaf chamber. Figure 6.10 depicts the experimental setup:

- A leaf chamber is typically made of transparent plastic with a variable internal volume which will be dependent on the leaf size. The chamber has two small air vents to allow ambient air to flow into the chamber and mix with the water vapor generated by transpiration from the leaf.
- A 1/8-inch ID Teflon tubing connects the leaf chamber to the analyzer.
- Place a leaf, which remains attached to the plant, into the leaf chamber.
- Ensure that the CRDS analyzer is calibrated for isotopic composition and concentration dependence of the isotopic composition. For information on how to calibrate the Picarro Water Isotope Analyzer refer to the User's Manual. A recent version is available at:
  https://picarro.box.com/s/0nh2wvm4n4ojf8jlmj7v.
- As detailed previously, operate the analyzer in the dual measurement mode: liquid and vapor. The liquid measurement allows the analyzer to calibrate itself with a liquid water standard while the vapor mode analyzes the sampled water vapor to provide isotopic composition and concentration.

## 7 Application of Other Isotopes

As mentioned in earlier sections of this chapter, nuclear and isotopic techniques have a wide range of applications in the soil-water-plant interaction studies, covering the fields such as plant ecology, physiology, biochemistry, nutrition, microbiology, protection against insect pests, and soil fertility, chemistry, physics, and hydrology, etc. Few common examples of the applications of isotopic and nuclear techniques in agricultural research are listed below.

- $^{32}$P fertilizer use efficiency, root activity, DNA probes in molecular biology
- $^{35}$S in soil and fertilizer studies
- $^{65}$Zn in plant uptake and use efficiency
- $^{13}$C, $^{14}$C in soil organic matter dynamics, root activity, photosynthesis, pesticide residues, water use efficiency, etc.
- $^{22}$Na, $^{36}$Cl, $^{40}$K in ion uptake and mechanism of salt tolerance in plants
- $^{137}$Cs in soil erosion studies
- $^{60}$Co for sterile insects in integrated pest management (IPM)
- $^{198}$Gold-198 for detection of termite colonies in agricultural fields

The nuclear and isotopic techniques are the supporting tools, and not substitute, to the conventional techniques for understanding the biological processes and mechanisms of ecosystem functioning. Therefore, a careful evaluation is required with regard to: i) the need for using an isotopic/nuclear technique, and ii) the choice of the appropriate isotopic/nuclear considering the research objective, facilities and expertise available, risks involved in safe handling and disposal of hazardous materials, and the financial considerations. In this context, the stable isotopes are the ever preferred choice in soil-water-plant-atmosphere studies. Thus, examples and protocols of using $^{15}$N, $^{18}$O and $^{2}$H in plant nutrient and water use efficiency studies have been elaborated in this chapter. The reader is, however, referred to the IAEA Training Manuals (IAEA 1990, 2001) and the review by Nguyen et al. (2011), may one need further details.

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