1. GENERAL INTRODUCTION

1.1. SALT AND SOLAR SALTWORKS

In one of the Hadith recorded in Sunan Ibn Majah, Prophet Muhammad is reported to have said that: “Salt is the master of your food. God sent down four blessings from the sky – fire, water, iron and salt”.

Common salt chemically known as sodium chloride (NaCl) is one of the most widely used substances on earth with ~14000 direct and indirect uses from food to industry to de-icing reported. The root of both words in the Latin sal (salt), which derives from the Greek words, sea and salt. The initial letter s in the Latin word derives from the early Greek whereby the words for salt and sea started with an s too. Later in ancient Greek, this s was dropped and was replaced by the breathing (Korovessis and Lekkas, 2000). The art of salt making in India has been carried on, from the time immemorial. In ancient time when seawater evaporated in pits, white layer was formed and it was found tasty and people started consuming it. The white layer was nothing but ‘salt’ (Jhala, 2009).

The pattern of solar salt production from the past to present has changed according to the surrounding environment and social situation. Modern solar saltworks are better and more stable ecosystems than the traditional ones (Korovessis and Lekkas, 2009). Some salt communities couldn’t adapt to the change and their salt customs have been devoured by trends of modern society, but
some salt communities have found ways to cope with the changes of globalization and adapted themselves in line with modern society (Sintusaard et al., 2009).

1.1.1. History of salt

Salt, the world’s best-known mineral made up of sodium (Na⁺) and chloride (Cl⁻) is the first substance after water to have attached human’s attention in their evolution from wilderness to civilization (Korovessis and Lekkas, 2009). Salt occurs naturally. It is found in the world’s oceans, inland waters, salt domes and sedimentary deposits. Solar evaporation is the easiest and oldest method of salt production. The food preserving property made salt one of the most important commodities for centuries. From primitive to modern times, every human being has had an association with salt. In ancient, Greece involving exchange of salt for slaves. Salt was used as payment during the Roman Empire. Roman soldiers were usually paid in the form of salt instead of money. The word salary is derived from the Latin word salarium. The term salarium itself derives from sal and can best be translated as salt-money (Reinhard, 1996). By 2000 BC, people know that adding salt to food stopped it from decay. The buying and selling of salt become one of the most important trading activities in the world. In many religions salt was given as a blessing. Salt means longevity, strength, trustworthy and intelligent (Mark, 2002). Salt is formed into crystals by the natural combination of sodium and chlorine. In India, salt played a very important role to give independence to our nation from British Kingdom. Mahatma Gandhi led at least 100,000 people on the “Dandi March” on “Salt Satyagraha” as it avoided paying the “salt tax”. In
Thailand, salt production from seawater practice since the beginning of Ayutthaya Era (Sintusaard et al., 2009).

1.1.2. Properties of salt

Salt is a biological requirement of human life. Various forms of salt are available today, viz. table salt, kosher salt, iodized salt, rock salt, sea salt, pickling salt, celtic salt and seasonal salt. Salt crystals are cubic in form and are often used as an example of crystalline structure. Chemically, it is 39.3% sodium (Na) and 60.7% chlorine (Cl) by weight and has been given mineralogical name ‘halite’. Atomic weight of elemental chlorine is 35.4527 and that of sodium is 22.989768. Salt is sold in several different particle sizes / gradations and forms depending on the intended use. The properties of salt are given in Table 1.1.

Table 1.1. Properties of Salt

<table>
<thead>
<tr>
<th>Chemical Properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Formula</td>
<td>NaCl</td>
</tr>
<tr>
<td>Atomic weight – Na</td>
<td>22.989768 (39.337%)</td>
</tr>
<tr>
<td>Atomic weight – Cl</td>
<td>35.4527 (60.663%)</td>
</tr>
<tr>
<td>Formula weight</td>
<td>58.44 g mol⁻¹</td>
</tr>
<tr>
<td>Colour</td>
<td>Clear white</td>
</tr>
<tr>
<td>Appearance</td>
<td>White crystals</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Physical Properties</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific gravity</td>
<td>2165 gr / cm</td>
</tr>
<tr>
<td>Critical humidity</td>
<td>75.3%</td>
</tr>
<tr>
<td>Melting point</td>
<td>801°C</td>
</tr>
<tr>
<td>Boiling point</td>
<td>1413°C</td>
</tr>
<tr>
<td>Refractive Index</td>
<td>1.544</td>
</tr>
</tbody>
</table>
1.1.3. Sources of sodium chloride

Salt is an important substance in the chemical industry and in food preparation, is produced from four main sources: lakes, seas, salt rock and underground saltwater. Salts from lakes has the lowest cost and is of high quality seawater has a lot of salt; it contains an average of 2.6% (by weight) NaCl, or 26 metric million tons per cubic kilometer (120 million short tons per cubic mile), an inexhaustible supply. Rock salt is found in the ground and it can be extracted either by drilling, blasting and hauling it to the surface (Anonymous, 2005). Underground salt deposits are found in both bedded sedimentary layers and domal deposits. Some salt is found on the surface as the dried-up residue of ancient seas, like the famous Bonneville Salt Flats in Utah (Ertem et al., 2001).

1.1.4. Solar saltworks – A complex ecosystem

Salt is produced by solar evaporation is the natural process, which requires water from the ocean, sunlight and wind. A series of connected shallow ponds where salinity gradient is well maintained throughout the ponds provide a favourable environment for the growth of variety of microorganisms in the water as well as on the floors (Moosvi, 2006). Saltworks are very special places for a variety of reasons because of the salt they produce and their historical, cultural and ecological values, as well as for their landscape: special, even extreme, at the edge of the earth, sea and air (Kavakli et al., 2006). Saltworks usually present important wetlands exceptionally rich in biodiversity. The ecological value of the saltworks stems from their shallow ponds whose floors are the storehouses for birds, shellfish and other animals (Dahm, 2004; Petanidou, 2004). The birds occupy these areas on
a seasonal basis, in keeping with their needs. It provides favourable factors like feeding, nesting and breeding sites and constant protection which directly or indirectly benefit the birds. Yet there is one more important aspect of solar saltworks which adds to the environmentally friendliness of the process (Moosvi, 2006). The solar saltworks as representative wetland ecosystems have high vulnerability due to natural processes and human interventions in the coastal zone (Davis, 2009).

Generally solar saltworks consists of three distinct biological areas (reservoir, condenser and crystallizer), which can be conveniently as a function of salinity. The first area includes ponds whose vary from that of seawater to approximately three times greater than its salinity. The second ranges from three to seven times and the last more than seven times salinity to saturation in respect to sodium chloride (Reginald, 2003). Saltpans are one of the hypersaline extreme environments. They are characteristically exposed to a wide range of environmental stress and perturbations manifest mainly through salinity changes (Nagasathy and Thajuddin, 2008). Salt is mainly a basic inorganic chemical raw material. In the field of inorganic chemistry solar salt production is truly a remarkable and uniquely efficient process (Sedivy, 2009).

1.1.5. Role of microorganisms in solar saltworks

Microalgae are the biological starting point of energy flow in most aquatic ecosystem and the basis of food chain (Bradach et al., 1972). Use of microalgae for human consumption is a source of high value health food, functional foods and for production of biochemical products (Borowitzka, 1992; Pough et al., 2001).
Microbial environments are highly productive, but during daytime most of the oxygen produced by the photoautrophs appear to be recycled within the algal mats rather than exchanged with the overlying water and the atmosphere. The growth of diatoms controlled by salinity, light penetration, nitrate concentration and they can be used as indicators of time-integrated water quality in the ponds of solar saltworks (Campbell and Davis, 2000). Fluctuations of salinity, water chemistry and nutrient dynamics seem to be the main constrains of biodiversity, food web structure and functional patterns in saline ecosystems (Golubkov and Golubkov, 2009).

Biological systems produced by microorganisms in every solar saltworks along the complete salinity range due to their importance are able to help or harm salt production (Davis, 1990, 2009; Pedros – Alio et al., 2000; Ayadi et al., 2004; Segal et al., 2006). The early studies by Davis (e.g. Davis, 1979; Davis and Giordano, 1996) have shown that active biological communities are essential for the proper functioning of salterns, but that too extensive development of the biota may lead to the production of poor quality salt. Sudden fluctuations in salinity and the other parameters frequently lead to a decrease in quality of salt that precipitates in the crystallizers.

The cause of the differences in crystallization behaviour of halite in the crystallizer ponds may be well connected with biological phenomena and with organic compounds (Sedivy, 2009, 2009a). Microorganisms (e.g. algae, bacteria, copepods, worms) suspended in the water (planktonic communities) aid salt production by colouring the water to improve solar energy absorption and water
evaporation. Microorganisms living on pond floors (benthic communities) seal ponds against water leakage and infiltration, permanently remove important quantities of combined nitrogen and phosphate from the overlying water, remain permanently attached to pond floors, and maintain desired thickness in all ponds (Davis, 1979; Javor, 2002; Jhala, 2006; Oren, 2010).

The principal salts that crystallize from concentrated brine along with sodium chloride are the chlorides and the sulphates of magnesium, calcium and potassium. The harvested salt in the form of wet crystals, washed with brine to remove insoluble matter as well as soluble impurities (Jhala, 2006).

1.1.6. Microbial activities in saltern ponds

The production of high quality sodium chloride from a seasonal solar saltworks depends mainly on the functioning of the brine biological system (Kavakli et al., 2006). Biodiversity is highest where specific gravity of brine is from 1.120 to 1.140. As salinity increases, gypsum deposition begin in thin, discontinuous crusts and individual crystals of calcium sulphate interspersed in the uppermost layer of the benthic communities and proceeds to develop firm sheets on the floors (Davis, 2009). The salinity increased there would be a reduced diversity in the microbial community (Benlloch et al., 1995; Casamayor et al., 2000). The uppermost yellow 1 mm to 2 mm to orange layer consists of bacteria, unicellular and filamentous cyanobacteria, ciliates, diatoms and dinoflagellates. The second 5 mm to 10 mm green layer contains unicellular and filamentous bacteria and nematodes. The third layer constitutes 1 mm to 2 mm red layer of purple bacteria. The lowermost layer of variable thickness (10 mm to 20 mm) is largely living and
dead bacteria, moribund cyanobacteria and black organic mud. The benthic communities favourable to development and maintenance of mats firmly attached to pond floors that sustain desired thickness, preserve diversity, contribute nutrient sequestration (Davis and Giordano, 1996) and control leakage (Davis, 2009; Zhiling and Guangyu, 2009) improve soil condition, brine purifying and sealing the bottom. The algal blooms increase solar heat absorption and aids rapid evaporation (Reginald et al., 2009a).

1.1.7. Salt in chemical industries

Around 20% of the international salt production is destined for human consumption, whereas 55% is used in the chemical industry and 15% for de-icing roads in winter, water treatment, production of cooling brines and many other similar applications (Korovessis and Lekkas, 2009; Sedivy, 2009).

The biggest consumer of salt is the chlor – alkali industry. The chlor-alkali sector is using salt for production of a large number of inorganic chemicals such as chlorine, sodium hydroxide (caustic soda), sodium carbonate (soda ash), sodium sulphate and hydrochloric acid. It also used in a number of industries, such as soap manufacture, textiles, regeneration of exchange resins and in the food industry such as canning of fish, meat and vegetables (John, 2004; Weerati, 2004; Mensah and Bayitse, 2006). Other uses are in the leather tanning industry, commercial dye manufacture and in the pulp and paper industry (Hough, 2008).

1.1.8. Consequences of salt

Salt is an inseparable ingredient, which sustains life on earth. Chloride and sodium are the two major components of salt are necessary for the survival of all
known living creatures. Our body requires salt to maintain tissue and muscle’s health. Too much or too little salt in the diet can lead to muscle cramps, dizziness or electrolyte disturbance, which can cause neurological problems, or be fatal. Salt used as a health aid for the treatment of dysautonomia.

Our body is composed of six thousand billion cells. Each cell floats in the solution of NaCl. Salt is involved in regulating the water content (fluid balance) of the body. Salt is used for preserving hides and skins, as well as in stock feeds. Sodium is restricted mainly to the extracellular fluid and potassium to the intracellular space, where both ions act to hold water and control fluid volume by osmotic activity. Excess salt is indigested and usually excreted by kidneys which regulate the amount of sodium in our body. Kidneys conserve sodium when levels are low and excrete excess amounts in the urine when levels become too high (Anonymous, 2005). Evaporation of sweat from exposed skin is the predominant mechanism for heat loss in humans. Physical exercise at hot temperature requires increased fluid and sodium intake (Titze, 2007). With the beginning of an agricultural society, humankind found the need to supplement vegetable and cereal diets with extra quantities of salt (Kostick, 1993). The annual human requirement of salt in temperate region is about 5 kg/year. In the tropics this is higher (Kaufmann, 1960). The physiological needs to salt in humans are as low as 1g/day nobody can live without salt (Drueke, 2007). High salt intake promotes hypertension (Taubes, 1998), cardiovascular mortality (Luft and McCarron, 1991), heart failure, stroke and chronic kidney diseases.
Experimental results indicate that salt deficiency stunts the growth of young animals. Salt deficiency for long time affects the dairy cows get hit the hardest many collapse and lead to die. Salt deficiency causes rough coat, decreased feed intake, licking and chewing various objects as well as decreased production. Salt has remained a necessary element for the survival and proliferation of not only herbivorous animals, which take the necessary quantity of salt by licking the salty soil, but also for carnivorous ones, which ensure the necessary intake of salt from the blood of their prey (Young, 1977).

1.1.9. World salt production

Global demand for salt is forecast to grow 2.5% per year to 305 million metric tons in 2013. At present, about 120 countries are actively engaged in salt production. Now India is the fourth largest salt producing country in the world after China, USA and Germany. The sea and the subsoil brine are the main sources of salt in India. Salt is produced along the coast in the states of Gujarat, Maharashtra, Goa, Karnataka, Tamil Nadu, Andhra Pradesh, Orissa and West Bengal. Gujarat is the major producer of salt followed by Tamil Nadu and Rajasthan. The average annual production of salt in Tamil Nadu in 2010 was about 14.5 lakh tons. In Tamil Nadu salt is produced in large quantities in the districts of Tuticorin, Nagapattinam, Ramnad and Kancheepuram. India exports salt both for human and industrial consumption. Exports are taking place mainly through sea transport. India is a traditional producer of salt from historical times. But the productivity in India is much lower. By increasing the productivity, the total production of salt is expected to reach 38 – 40 million tons by 2020. The country
has potential to increase production, productivity and quality (Varmudy, 2010). Recently, the annual world production of salt exceeded 200 million tons.

Table 1.2. World Salt Producers and Reserves

<table>
<thead>
<tr>
<th>Countries</th>
<th>Salt production (in million tons)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2009</td>
</tr>
<tr>
<td>China</td>
<td>59,500</td>
</tr>
<tr>
<td>USA</td>
<td>46,000</td>
</tr>
<tr>
<td>Germany</td>
<td>16,400</td>
</tr>
<tr>
<td>India</td>
<td>16,000</td>
</tr>
<tr>
<td>Canada</td>
<td>14,400</td>
</tr>
<tr>
<td>Australia</td>
<td>11,000</td>
</tr>
<tr>
<td>Mexico</td>
<td>8,810</td>
</tr>
<tr>
<td>Brazil</td>
<td>6,900</td>
</tr>
<tr>
<td>Chile</td>
<td>6,430</td>
</tr>
<tr>
<td>France</td>
<td>6,100</td>
</tr>
<tr>
<td>World total (including others)</td>
<td>2,80,000</td>
</tr>
</tbody>
</table>


1.1.10. Scope and aim of the present study

Salt has often been considered as an extremely valuable commodity throughout the ages of human history because of its importance to survival. In the point of view on quality specification the country is losing a lot of money on purification and refining process which affects the country’s economy. Hence apart
from manufacturing crude salt in saltpans, the quality is to be maintained. Since proper biological management of solar saltpans makes sure high quality salt, the present work was undertaken to increase salt quality by inoculating halotolerant, unicellular green alga *Dunaliella salina* in the solar saltworks.

1. The present work includes the analysis of physical and chemical parameters of the brine samples present in the various stages of salt production process (reservoir, condenser and crystallizer) in Puthalam saltworks.

2. To list out the microalgae present in various ponds of saltworks and also calculate the abundance of phytoplankton.

3. To estimate the biomolecules such as chlorophyll ‘a’ and total protein in the algal samples of various concentrations in the selected saltworks.

4. To carry out the salt quality analysis for the salt quality parameters like moisture, insoluble matter, sulphate, calcium, magnesium and sodium chloride content of the salt samples harvested from *Dunaliella salina* inoculated ponds (experimental) and uninoculated ponds (control) in this saltworks to reveal the role of beneficial microalgae in quality salt production.
1.2. GENERAL MATERIALS AND METHODS

1.2.1. Description of the study area

In Kanyakumari District salt is produced in around 600 acres with an estimated annual production of 0.11 lakh tons. Here, eight saltpan industries are involved in the salt production process. In these, the typical tropical saltworks Sri Sankara Allom Salt Factory, Puthalam, Kanyakumari District (Plate 1) in South Tamil Nadu is situated between Manakudy seacoast and Puthalam village. Puthalam village is 12 km away from Nagercoil town (Map 1). This allom was the first to use sub-soil brine for salt production. It had its beginning in 1911 A.D. Salt and gypsum are manufactured in this saltworks. This saltworks is also close to the Manakudy estuary, but the estuarine water is not used for salt production. Instead, the source sub-soil brine is pumped from underground into a big pond which acts as the reservoir (Plate 1a). Water is used when it is needed from the reservoir. The salinity gradually increases by solar evaporation as water flows from one evaporator to another. After the brine becomes saturated, it is transferred to crystallizing pans. The layout of this allom (saltworks) is ideal. The total area of this salt work is 24.88 ha. The average time taken to harvest salt is 35 to 40 days. It is situated 13 km south east of Nagercoil and 5 km north of Kanyakumari at 8° 04’ N latitude and 77° 68’ east longitude. This is one of the best salt producing area in Kanyakumari District (Plate 1e) and also contains rich plankton community. Low salinity ponds of Puthalam saltworks attract large number of migratory birds and form an important feeding and roosting area (Plate 1f). One of the most
important features of this allom is that the salt crystal is big, specially manufactured for use in chemical industry.

1.2.2. Seasons of the study period

For this study, the two years investigation work was carried out from March 2009 to February 2011. The first year study period began from March 2009 to February 2010 and the second year study period commenced from March 2010 to February 2011. This study period (from March 2009 to February 2011) was divided into four seasons viz. summer (March, April and May), autumn (June, July and August), spring (September, October and November) and winter (December, January and February).

1.2.3. Phases of salt production

Based on brine concentration (salinity) the phases or stages of solar salt production process is divided into three different stages and occurred in reservoir, condenser and crystallizer (Plates 1b, c and d).

1.2.4. Physico-chemical parameters

The physico-chemical parameters such as atmospheric temperature, brine temperature, brine pH, depth of the ponds, brine density (salinity), biological oxygen demand, total dissolved solids, chloride, sulphate, sodium, calcium, iron, magnesium and potassium were determined four times for one season and four readings were taken for each variables.
1.2.5. Plankton and classification

In biology, detritus is non-living particulate organic material typically dead organisms colonized by microorganic communities. Phytoplanktons are the autotrophic component of the plankton community and they fix solar energy by photosynthesis, using carbon-dioxide, trace elements and nutrients. They appear as green due to the presence of chlorophyll. The colour may vary due to various levels of chlorophyll or the presence of accessory pigments such as phycobiliproteins, xanthophylls etc.

Plankton may be arbitrarily classified by size as nanoplankton (cells < 20 µm), microplankton (between 20 and 200 µm), mesoplankton, macroplankton and megaplankton (above 200 µm) (Lenz, 1968; Sourhia, 1968; Lokhande and Shembekar, 2009). Fine mesh nets (Millipore filter) are used for capturing phytoplankton, which can be extremely useful for morphological and taxonomical studies, because net hauls provide large number of certain species, which are generally sampled infrequently by pre-cleaned water bottles and stored in air tight pearl pet container labeled with details.

1.2.6. Bulk measurements

Taking quantitative data of the standing of phytoplankton, bulk measurements like particulate carbon, nitrogen or phosphorus and chlorophyll are applicable (Strickland and Parsons, 1972). Microscopic observation is the only means to identify and count the phytoplankton at the species level till recently. The important biomolecules of microalgae such as chlorophyll ‘a’ and total protein were estimated by standard methods.
1.2.7. Microalgae identification and culture

Microalgae in various ponds of the Puthalam saltworks were taken and observed under the microscope. Photographs were taken by using a digital camera (Pentax - 6.0 megapixels). Then they were identified, based on standard monographs (Venkataraman, 1939; Prescott, 1962; Desikachary, 1986). Name, systematic position and the abundance of the microalgae were tabulated.

For biotechnological use, a single algal cell was isolated by serial dilution and cultured by using the standard Walne’s medium. Basically Walne’s medium consists of solution A, B, C and D and it is prepared by following methods.

1.2.8. Preparation of Walne’s medium

Solution A prepared by dissolving potassium nitrate (100 g), sodium ortho-phosphate (20 g), EDTA (45 g), boric acid (33.4 g), ferric chloride (1.3 g) and manganese chloride (0.36 g) in one litre distilled water.

Likewise, solution B prepared by dissolving zinc chloride (4.2 g), cobalt chloride (4 g), copper sulphate (4 g) and ammonium molybdate (1.8 g) in one litre distilled water.

For solution C, 200 mg Vitamin B₁ (thiamine) dissolved in 100 ml distilled water. Similarly, 10 mg of Vitamin B₁₂ (cyanocobalamin) dissolved in 100 ml distilled water for Solution D.

Walne’s medium was prepared by mixing all the solutions in the proper proportion. For one litre Walne’s medium 1 ml of Solution A, 0.5 ml of Solution B, 0.05 ml of Solution C and 0.05 ml of Solution D were mixed in one litre
sterilized seawater. Much quantity of Walne’s medium was prepared depend on the need for the culture of microalgae *Dunaliella salina*.

### 1.2.9. Salt quality parameters

Salt samples were taken for every season and the quality parameters such as moisture, insoluble matter, sulphate, calcium, magnesium and sodium chloride content were estimated and they were tabulated.

### 1.2.10. Statistical Analysis

The data obtained in this work have been subjected to the following statistical analysis.

#### 1.2.10.1. Standard Deviation (SD)

\[
SD = \frac{\sum d^2}{N - 1}
\]

Where, ‘d’ refers to the deviation of each score from mean and ‘N’ the total number of samples.

#### 1.2.10.2. Student’s ‘t’ test

Student’s ‘t’ test was used to compare two means.

\[
t = \frac{X_1 - X_2}{\sqrt{SE_1^2 + SE_2^2}}
\]

Where, \(X_1\) and \(X_2\) represent the means compared and \(SE_1\), \(SE_2\) are their respective standard errors. Standard error was calculated from the formula.

\[
SE = \frac{SD}{\sqrt{N - 1}}
\]
The level of significance for the ‘t’ at corresponding degrees of freedom (df) was read from the f distribution table given by Zar (1974).

\[ \text{df} = (N - 2) \]

Where, \( N \) is the total number of scores in both the experiments.

### 1.2.10.3. Two way Analysis of Variance

Partitioning of total variance into variance due to different experimental factors and their interaction was carried out following the procedure described by Zar (1974). Values obtained at the different experimental conditions were tabulated in different columns and rows. For each column, \( X \) and \( X^2 \) were calculated. Sum of \( X \) for all the columns squared are divided by the number of tabulated values and correction factor ‘C’ was obtained.

\[
C = \frac{\text{Sum of all } X^2}{N}
\]

\( (N = \text{total number of observations}) \)

Total Sum of Square (TSS) = Sum of \( X^2 \) for all columns – C

Between Column SS (CSS) = \( \frac{\text{Sum of values in each column } X^2}{\text{Number of values in the columns}} \)

Between row SS = \( \frac{\text{Sum of all values in each row } X^2}{\text{Number of values in the rows}} \)

Remainder SS = Total SS – (Between columns SS + Between rows SS).

Considering the degrees of freedom for each source of variance, mean sum of square (MS) was calculated.
**Degrees of freedom (DF)**

Total DF = Number of valuables in table – 1.

Between columns DF = Number of columns – 1.

Between rows DF = Number of rows – 1.

Error DF = Total DF – (Between columns DF + Between rows DF)

**F value for the variance**

Between columns = \[rac{MS \text{ between columns}}{Error \ MS}\]

**F value for the variance**

Between rows = \[rac{MS \text{ between rows}}{Error \ MS}\]

Significant level (5% level) at the corresponding DF was read from standard tables.