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Fourier-Transform Infrared Spectroscopy as an aid for The determination of Cyanobacteria in surface water In Jericho.

By:

Nuha Hijazi Mohammad Hijazi.

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Prepared by:

Nuha Hijazi Mohammad Hijazi.

B.Sc. Biology and Medical Technology,

College of Science and Technology-Palestine

Supervisor: Dr. Mutaz Qutub

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Faculty of science and Technology-Al-Quds University.

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Dedication:

To my dear husband, my parents, my mother in law.

My children.

My teachers.

All of my friends.

Declaration:

I certify that this thesis submitted for the degree of Master is the result of my own Research, except where otherwise acknowledged, and that this thesis (or any part of The same) has not been submitted for a higher degree to any other university or Institution.

Signed……..

Date:

Nuha Hijazi Mohammad Hijazi.

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Abstract:

The aim of this investigation is to study the possibility of using FTIR spectroscopy for monitoring water quality.

Water samples from three sampling sites were collected; monthly samples around the year (January 2006 till January 2007) were collected from site I (Jericho Aquaculture pond) one sample from site II (Jericho agriculture pond). While one sample was collected from sampling site III which was Sulaiman pond.

All of these samples have been screened by the microscope for any presented microorganisms. Microscope results showed that all water sample collected from site I and site II contain Oscillatoria (cyanobacteria), which was dominant all over the year in site I and in site II. This dominance is mainly due to high nutrient levels in Jericho aquaculture in addition to the environmental conditions that are suitable for this microorganism. In the water sample that was collected from site III no Oscillatoria was presented, but few types of invertebrates and other types of microorganisms.

To check the validity of FTIR in determining the type and amount of these microorganisms all water samples have been checked by FTIR .

Results of FTIR showed Oscillatoria fingerprint in all samples collected from site I and site II. A different fingerprint was obtained for sample from site III.

A standard of Oscillatoria was prepared to compare results with, Oscillatoria standard spectrum was identical to all spectra of samples collected from site I and site II, but completely different fingerprints for site III.

In the quantitative test: in order to study the optimization of FTIR in Quantitative analysis of microorganisms. The same water sample that was containing Oscillatoria was exposed to sunlight to enhance Oscillatoria growth for one week. Daily water sample with about 100ml volume of water was filtered. All dry samples collected on the filter paper were milled and prepared as Kbr disks for FTIR analysis. FTIR showed that when the quantity of Oscillatoria was increased (proliferation enhanced by continues exposure to sunlight) the absorbance was increased (Transmittance was decreased) which reflect the suitability of FTIR in quantitative determination of these microorganisms.

Various porosity filter papers was used in another investigation the effect of the porosity of filter paper on the diversity of microorganism presented in water samples. Results showed that Oscillatoria fingerprints were clearly identified. The results of these investigations show that FTIR is very accurate in determination of water quality because it can determine type and amount of microorganism found in water, early determination of water quality will lead to sustainable critical water resources. A rapid inexpensive method for water quality determination like FTIR is strongly recommended.

ملخص:

تهدف هذه الدراسه الى التاكد من امكانية استحدامFTIR في دراسة جودة المياه .

في هذا البحث تم تجميع عينات ماء من ثلاث مناطق ، حيث تم جمع عينات شهرية على مدار العام بداية من كانون الثاني عام 2006 وحتى كانون الثاني 2007 من الموقع الاول بركة في منطقة اريحا وتم جمع عينه من الموقع الثاني وهو عين السلطان في اريحا ايضا كذلك تم جمع عينة واحدة من الموقع الثالث وهو برك سليمان في منطقة الخضر.

تم فحص جميع العينات بداية باستخدام المجهر وذلك من اجل تحديد أنواع الكائنات الحية الموجودة في عينات الماء ، وقد اظهرت نتائج الفحص المجهري أن الكائن الحي السائد على مدار العام في العينات التي تم جمعها من الموقع الاول هو نوع واحد من الطحالب الخضراء المزرقة (airotallicso) وكذلك الامر في عينات الماء التي تم جمعها من الموقع الثاني و السبب في ذلك يعود لإرتفاع (nutrient level) . أما العينة التي تم جمعها من الموقع الثالث فلم تكن تحتوي على هذا النوع من الطحالب الخضراء المزرقة ولكنها كانت تحتوي على عدة أنواع من الكائنات الحية الدقيقة.

من اجل فحص ما اذا كان FTIR يستطيع تحديد نوع و كمية الكائنات الحية الموجودة في عينات الماء تم تحضير جميع العينات لتحليلها باستخدام FTIR . نتائج FTIR اظهرت ان جميع العينات التي تم تجميعها من الموقع الاول و الثاني لها نفس الطيف اما العينة التي جمعت من الموقع الثالث فقد كان طيفها مختلفا .

لمعرفة ما اذا كان FTIR يستطيع تحديد كمية الكائنات الحية الموجودة في عينة الماء تم وضع عينة ماء تحتوي على (oscillatoria) في الشمس من أجل تكثير هذه الطحالب الخضراء المزرقة ، و تم ترشيح نفس الكمية من العينة قبل و بعد وضعها تحت اشعة الشمس لمدةاسبوع وتمت مقارنة الطيفين . أظهرت النتائج ان شدة الامتصاص بعد اسبوع من وضع العينة تحت اشعة الشمس ازدادت مما يدل على ان FTIR يحدد ايضا كمية الكائنات الحية الدقيقة الموجودة في عينة الماء و ليس فقط نوعها .

تم تغير نوع ورقة الترشيح المستخدمة في ترشيح عينات الماء فقد تم استعمال ورقة ترشيح ذات ثقوب كبيرة و اخرى ذات ثقوب صغيرة في دراسة لاحتمالية تداخل الاطياف في حالة احتواء عينة الماء على اكثر من نوع من الكائنات الحية وما اثر ذلك على طيف (oscillatoria) .أظهرت النتائج ان لا تداخل في الاطياف و ظهر طيف (oscillatoria) بوضوح مما يعني ان جهاز FTIRدقيق في تحديد جودة المياه من خلال تحديد نوع و كمية الكائنات الحية الموجودة فيها و التي تعكس وضع الماء الكيميائي، أن الكشف المبكر لجودة المياه يساعد على الحفاظ على مصادر المياه . بناءا على ما تقدم فان استخدام FTIR في دراسة جودة المياه أمر اوصت به الدراسة بشدة.

Abbreviations and Units

Abbreviation:

T%	Transmittance.
IR	Infra Red.
FTIR	Fourier Transform Infra Red Spectroscopy.
WHO	World Health Organization **.**
U.S EPA	United State Environmental potential agency.
ATP	Adenosine Tri Phosphate.
PCR	Polymirase Chain Reaction.
Vs	versus.

Units:

mg	Milligram.
cm^{-1}	reciprocal Centimeter.
L	litter.
Km^2	Killometer square.
M^2	meter square.
M^3	cubic meter.
PCI	Pounds per Cubic Inches.

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Chapter 1

Introduction

Damage to the overall condition of aquatic resources due to anthropogenic influence, is becoming a serious problem, whereby an increase in pollutant levels is associated with a decrease in quantity and quality of water resources (Cary, 1972, Peterson, 1999). Knowledge of the state of water quality in aquatic ecosystems and the changes produced by human activities is the first step towards establishing an efficient water management system, as is essential for the preservation of these ecosystems. Biological indicators for monitoring water quality were introduced early in the 20^{th} century (Doulerelo , et al.,2003). A range of methods has been used to monitor water quality, including those employing algae , which have been widely used as bioindicators because it is very sensitive to ecosystem conditions(Graham , et al.,2000 , Bartram , et al ,1996). The most common are:

X-ray crystallography:

When membrane proteins have been exposed to x-ray crystallography they will be solved. It is very difficult to crystallized membrane proteins, not more than fifteen membrane proteins that have been already crystallized (Arora and Tamm ,2001).

Nuclear magnetic resonance (NMR) spectroscopy:

This method is limited to proteins of low molecular weight. It is unsuitable for detection of membrane proteins because they have large molecular weight (Arora and Tamm, 2001).

Fourier Transform Infrared spectroscopy (FTIR):

Direct proteins detection to determine different types of microorganisms that are founds in water cannot be used by common visible flourophores because of the high background fluorescence of the membrane. Using near infrared (670-1100nm) can be used effectively for direct protein detection, longer wave length provide excellent signals to noise ratio so there is no back ground fluorescence (Dudley, et al.,1987) . FTIR spectroscopy is a useful technique in the study of proteins especially membrane proteins (large proteins in turbid suspension). FTIR spectroscopy was first applied to proteins as early as in 1952 before any detailed X-ray results were available (Arrondo and Goxi, 1999). It has considerable potential for the study of

environmental samples allowing high-resolution analysis of single microorganism from another by its special spectrum. (Sigee, et al, 2003 , Mizaikoff , 2003) Infrared imaging visualizes the lipid and protein content of the cells and can be of great interest in the future (Arrondo, et al., 1999).

1.1 Optical spectroscopy:

Spectroscopy is defined as the study of interaction of electromagnetic radiation with matter, excluding chemical effects.

When an electromagnetic wave encounters a molecule, it can be either scattered, its direction of propagation changes or absorbed, its energy is transferred to the molecule. If the electromagnetic radiation of the light is absorbed, the molecule is said to be excited. An excited molecule can posses any one of a set of discrete amounts (quanta) of energy described by the laws of quantum mechanics. These amounts are called the energy levels of the molecule. The major energy levels are determined by the possible spatial distributions of the electrons and are called electronic energy levels; on these are superimposed vibrational levels, which indicate the various modes of vibration of the molecule. All these energy levels are described by an energy-level diagram (Figure 1).

The lowest electronic level is called the ground state and all others are excited states .

For most purposes, it is convenient to treat a molecule as if it possesses several distinct reservoirs of energy. The total energy is given by the following equation (Kemp,1987)

$$E_{total} = E_{transition} + E_{electronic} + E_{rotation} + E_{vibration} + E_{electron\ spin\ orientation} + E_{nuclear\ spin\ orientation}$$

Energy can effect the molecules in a number of forms, of which the most important ones, are transnational, electronic, vibrational, and rotational energy. Infrared spectroscopy is based on molecular vibrations and monitors the transition between vibrational energy level.

Light is a electromagnetic wave. The energy of the wave is:

$E=h.c/\lambda .=h.v$ (Dudley, et al.,1987)

In which h is Planck's constant, c is the velocity of light, λ is the

Wavelength and v is the frequency.

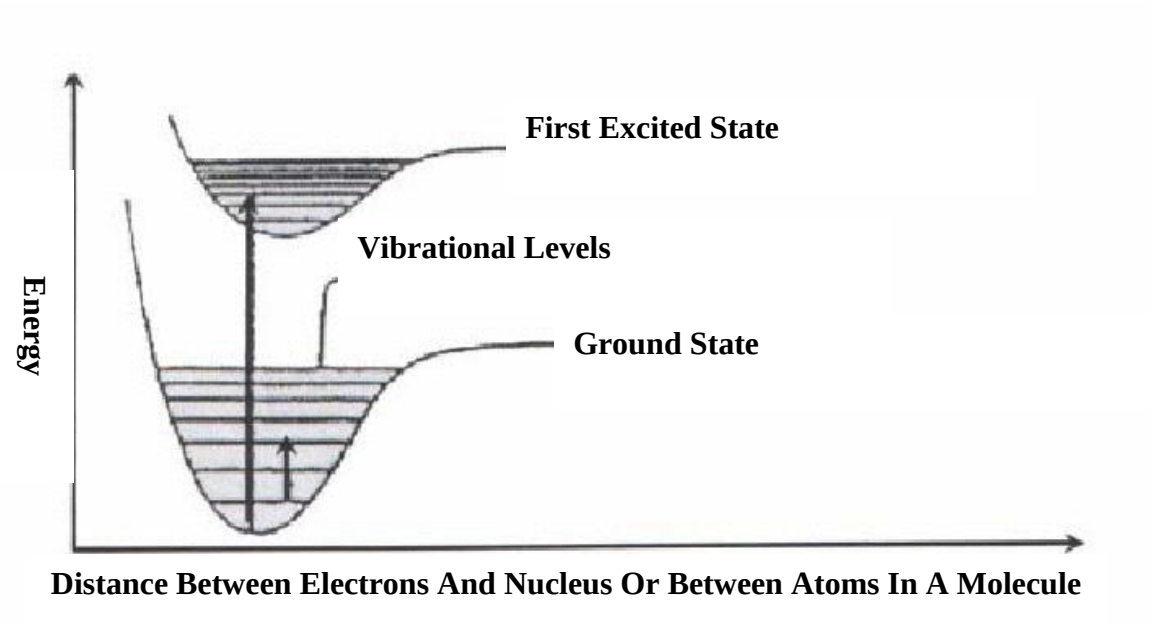

Figure 1:Typical energy-level diagram showing the ground state and the first excited state (Sherman,1994).

Vibrational levels are shown as thin horizontal lines. A possible electronic transition between the ground state and the fourth vibrational level of the first excited state is indicated by the long arrow. A vibrational transition within the ground state is indicated by the short arrow

1.2 Fourier Transform Infrared (FTIR) Spectroscopy

An infrared spectrum has come into use recently. Light covering the whole frequency range, typically 5000-400 $cm^{-1}$is split into two beams (Priyangika, et al., 2006). Either one beam is passed through the sample, or both are passed, in a Fourier Transform Infrared Spectrometer, a continuum source of light is used to produce light over a broad range of infrared wavelengths (Dudley, et al., 1987). Light coming from this continuum source is split into two paths using a half-silvered mirror; this light is then reflected from two mirrors back onto the beam splitter, where it is recombined (Remy, et al., 2004). One of these mirrors is fixed, and the second is movable. If the distance from the beam splitter to fixed mirror is not exactly the same as the distance from the beam splitter to the second mirror, then when two beams are recombined, there will be a small difference in the phase of the light between these two paths (Ross, et al., 1999). Because of the superposition, principle constructive and destructive interference exist for different wavelengths depending on the relative distances of the two mirrors from the beam splitter (Figure 2). In FTIR spectroscopy, the light is directed onto the sample of interest and

the intensity is measured using an infrared detector. The intensity of light striking the detector is measured as a function of the mirror position, and this is then Fourier-transformed to produce a plot of intensity vs. wave number (Sherman, 1994).

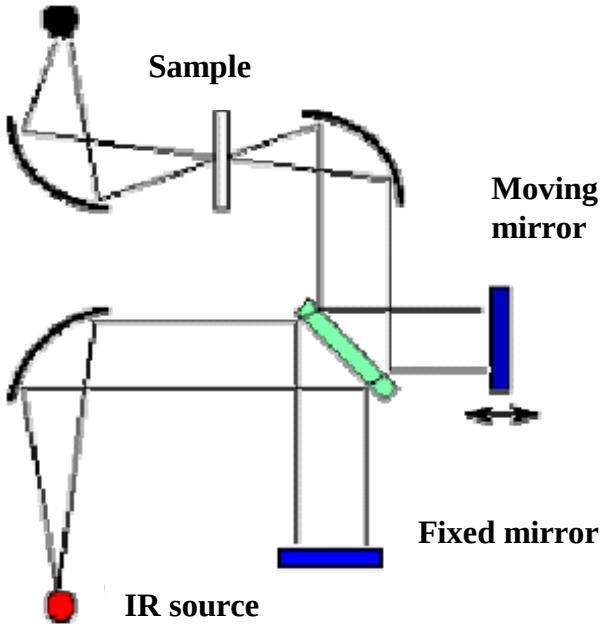

Figure 2:Schematic representation of FTIR spectrometer (Sherman,1994).

1.2.1 IR frequency range and spectrum presentation:

Infrared radiation spans a section of electromagnetic spectrum having wave numbers from 13.000 to 10 cm^{-1} or wavelengths from 0.78 to 1000 μm (Kemp,1987).

It is bound by the red end of visible region at high frequencies and the microwave region at low frequencies.

IR absorption positions are generally presented as either wave numbers (cm^{-1}) or wavelengths (λ). IR absorption information is generally presented in the form of a spectrum with wavelength or wave number at the x-axis and absorption intensity or percent transmittance at the y-axis. Transmittance, T, is the ratio of radiant power transmitted by the sample (I) to the radiant power incident on the sample (I0). Absorbance (A) is the logarithm to the base 10 of the reciprocal of the transmittance (T). The transmittance spectra provide better contrast between intensities of strong and weak bands because transmittance ranges from 0 to 100% Transmittance whereas absorbance ranges from infinity to zero (Dudley, et al., 1987). Then IR spectroscopy is the measurement of the wavelength and intensity of the absorption of infrared

light by a sample. Infrared light is energetic enough to excite molecular vibrations to higher energy levels. The IR region is commonly divided into three smaller areas: near IR, mid IR, and far IR.

Table 1:Infra red region (Amy, et al.,1998)

Region	Wavenumber(cm^{-1})
Near	**14000-4000**
Middle	**4000-400**
Far	**400-4**

1.2.2 Molecular vibrations

At the ordinary temperatures, organic molecules are in a constant state of vibration, each bond having its characteristic stretching and bending frequency, and being capable of absorbing light of that frequency (Kemp, 1987). Infrared spectra are generated by the characteristic twisting, bending, rotating and vibrational motions of atoms in a molecule (Walt, 1999). All of the motions can be described in terms of two types of molecular vibrations. One type of vibration, a stretch, produces a change of bond length. A stretch is a rhythmic movement along the line between the atoms so that the interatomic distance is either increasing or decreasing. The second type of vibration, a bend, results in a change in bond angle. These are also called scissoring, rocking or wigwag motions. Each of these two main types of vibration can have variations. A stretch can be symmetric or asymmetric. Bending can occur in the plane of the molecule or out of plane; it can be scissoring, like blades of a pair of scissors, or rocking, where two atoms move in the same directions (Figure 3).

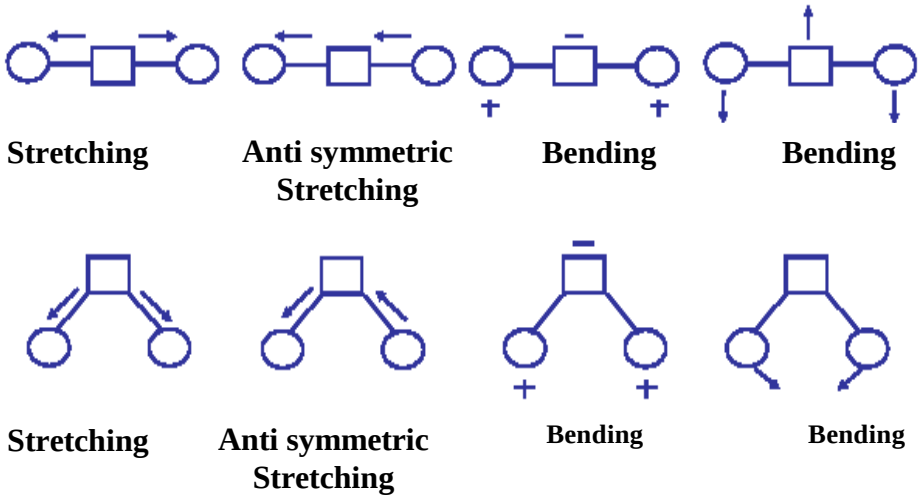

Figure 3 :Vibration modes in methylene groups. Similar AX2 groups(-NH2, -NO2,etc.)(Kemp,1987)

1.2.3 Spectrometer Components

There are three basic spectrometer components in an FT system: radiation source, interferometer, and detector. However, the source is more often water-cooled in FTIR instruments to provide better power and stability (Sherman, 1994). FTIR spectrometer differentiates and measures the absorption at component frequencies. The monochromator is replaced by an interferometer, which divides radiant beams, generates an optical path difference between the beams, then recombines them in order to produce repetitive interference signals measured as a function of optical path difference by a detector. The interferometer produces interference signals, which contain infrared spectral information generated after passing through a sample (Dudley, et al.,1987).

The most commonly used interferometer is a Michel son interferometer. It consists of three active components: A moving mirror, a fixed mirror, and a beam splitter (Kemp, 1987).

The two mirrors are perpendicular to each other. The beam splitter is a semi-reflecting device and is often made by depositing a thin film of germanium onto a flat KBr substrate. Radiation from the broadband IR source is collimated and directed into the interferometer, and impinges on the beam splitter. At the beam splitter, half the IR beam is transmitted to the fixed mirror and the remaining half is reflected to the moving mirror(Dudley, et al.,1987). After the divided beams are reflected from the two mirrors, they are recombined at the beam splitter. Due to

changes in the relative position of the moving mirror to the fixed mirror, an interference pattern is generated. The resulting beam then passes through the sample and is eventually focused on the detector. Differences in the optical paths between the two split beams are created by varying the relative position of moving mirror to the fixed mirror. If the two arms of the interferometer are of equal length, the two split beams travel through the exact same path length (Kemp, 1987). The two beams are totally in phase with each other; thus, they interfere constructively and lead to a maximum in the detector response. This position of the moving mirror is called the point of zero path difference (ZPD). When the moving mirror travels in either direction by the distance λ /4, the optical path (beam splitter–mirror–beam splitter) is changed by 2 (λ /4), or λ/2. The two beams are 180° out of phase with each other, and so interfere destructively (Sherman,1994). As the moving mirror travels another λ /4, the optical path difference is now 2 (λ/2), or λ. The two beams are again in phase with each other and result in another constructive interference. When the mirror is moved at a constant velocity, the intensity of radiation reaching the detector varies in a sinusoidal manner to produce the interferogram output (Kemp, 1987). The interferogram is the record of the interference signal. It is a time domain spectrum and records the detector response changes versus time within the mirror scan. If the sample happens to absorb at this frequency, the amplitude of the sinusoidal wave is reduced by an amount proportional to the amount of sample in the beam (Dudley, et al., 1987)

1.2.4 Theory of Infrared Absorption

At temperatures above absolute zero, all the atoms in molecules are in continuous vibration with respect to each other. When the frequency of a specific vibration is equal to the frequency of the IR radiation directed on the molecule, the molecule absorbs the radiation (Sherman, 1994). Each atom has three degrees of freedom, corresponding to motions along any of the three Cartesian coordinate axes (x, y, z). A polyatomic molecule of n atoms has $3n$ total degrees of freedom. However, 3 degrees of freedom are required to describe translation, the motion of the entire molecule through space, and 3 degrees of freedom correspond to rotation of the entire molecule. The remaining $3n - 6$ degrees of freedom are true, fundamental vibrations for nonlinear molecules. Linear molecules possess $3n - 5$ fundamental vibrational modes because only 2 degrees of freedom are sufficient to describe rotation. Among the $3n - 6$ or $3n - 5$ fundamental vibrations (also known as normal modes of vibration), those that

produce a net change in the dipole moment may result in an IR activity and those that give polarizability changes may give rise to Raman activity (Kemp, 1987). Naturally, some vibrations can be both IR- and Raman-active. The total number of observed absorption bands is generally different from the total number of fundamental vibrations. It is reduced because some modes are not IR active and a single frequency can cause more than one mode of motion to occur, additional bands are generated by the appearance of overtones (integral multiples of the fundamental absorption frequencies) (Sherman, 1994). Combinations of fundamental frequencies, differences of fundamental frequencies, coupling interactions of two fundamental absorption frequencies, and coupling interactions between fundamental vibrations and overtones or combination bands. The combination and blending of all the factors thus create a unique IR spectrum for each compound (Dudley, et al., 1987).

Chapter Two

Algae as biological indicators for aquatic ecosystems

2.1 Algae definition

Algae are aquatic organisms that are photosynthetic; oxygenic that is typically smaller and less structurally complex than land plants. Algae range in size from tiny single-celled species one micrometer in diameter to giant seaweeds over 50 meters long (Hoek, etal.,1995).

2.2 Algae as bioindicator:

A bioassay is a procedure that uses organisms and their responses to estimate the effects of physical and chemical agents in the environment. Bioindicators provide early warning of possible environmental deteriorations, and may provide sensitive measures of pollution (Graham, et al., 2000). One common use of algal cultures is as bioindicators in the detection, in natural or effluent waters, of algal nutrients or substances that are toxic to algae (Hoek, et al., 1995). While daphnids and fish have traditionally been the primary bioindicator organisms in aquatic ecosystems, algae are more sensitive than animals to some pollutants (Graham, et al., 2000).

2.2.1 Micro algae

Micro algae are divided into two main groups:

1- Prokaryotes - are cells that do not have a nucleus or many other types of organelles found in eukaryotes. This group contains the blue-green algae (Cyanobacteria) and other bacteria

2- Eukaryotes: are the cells of all other organisms containing nuclei and other organelles. All other Kingdoms are placed within this group (Protists, Fungi, Plants, and Animals).

2.2.2 Algae Ecology

Algae are a diverse group of organisms that survive in all different types of habitats. From the dry desert, to the Arctic Circle, to boiling springs these organisms have found a way to extract enough from their environment to live in even the harshest surroundings (Graham, et al., 2000). They range in size

from microscopic like phytoplankton to macro algae which reach meters in length and in complexity from single-celled to complex organisms that would rival even large plants. Algae are one of the first steps of the food web, they provide food for all types of animals including fishes, insects, some animals and humans (Douterelo, et al., 2003).

2.2.3 Cyanobacteria

Cyanobacteria also known as blue green algae were the dominant forms of life on earth for more than 1.5 billion years (Roberto, 1998). They were the most ancient oxygen-producing photo synthesizers (Datta, et al., 2004). Modern cyanobacteria are recognized for thief ability to occupy extreme habitats and valued for their ability to fix atmospheric nitrogen, bind and enrich soils, and produce medicinally useful compounds (Graham, et al., 2000). Cyanobacteria are of concern when they form blooms, especially when they produce toxins (Hoek, et al., 1995). Cyanobacteria are classified into seven orders (Table 2) shows these orders with their morphology.

2.2.4 Cyanobacteria History

The oldest fossils attributed to cyanobacteria are 3.5 billion years old remains from the Apex Basalt, a geological deposit in Western Australia (Graham, et al., 2000). The Apex fossils include a variety of types of multicellular filaments that resemble certain modern cyanobacteria such Oscillatoria as well as some modern non-photosynthetic bacteria. (Hoek ,et al.,1995).

2.2.5 Cyanobacteria ecology

A number of cyanobacteria species can tolerate temperatures as high as 72 C^{o} and occur in the cold deserts of Antarctica (Robert, et al., 2004). Cyanobacteria have unparallel abilities to survive long periods of desiccation (Douterelo, et al., 2003). Cyanobacteria also are found at higher altitudes, where summer high temperatures reach only about 0 C^{o}, and winter temperatures drop to –60 C (Graham, et al., 2000). Cyanobacteria can live in rock fissures and cracks and in cavities occurring in porous transparent rocks such as sandstone, granite, and marble, but not dense dark volcanic rocks (Hoak, et al., 1995).

Cyanobacteria also form part of the soil surface communities known as cryptogamic crusts, which occur over large areas of arid and semiarid land around the world(Graham ,et al.,2000).Cyanobacteria are more common in desert crusts (on soil of pH 8-9)than they are in certain grasslands, where soil pH may be more acidic (Hoak,et al.,1995).

Cyanobacteria are among the few organisms that can occupy high temperature aquatic environments, including hot springs and thermal pools. In alkaline and neutral hot springs and streams flowing from them, cyanobacteria can form thick, colorful mats that exhibit banding patterns representing the distribution of species with different temperature tolerances (Douterelo,et al.,2003).Another type of extreme habitat tolerated by several cyanobacteria is hypersaline water(varying salinities considerably higher than seawater).These include saline lakes, hypersaline marine lagoons, and solar evaporation ponds. Certain cyanobacteria species (Synechoccus-like form) can survive and remain metabolically active within crystalline salt deposits for long as ten months (Graham ,et al.,2000).

Table 2:Classification of cyanobacteria (Hoak,et al.,1995)

Classification of Cyanobacteria			
Morphology	Reproduction	Order	Names (general)
Unicellular or Colonial	Binary fission =splitting in two	Chroococcales	Gloeobacter Chroococcus Microcystis Synechocystis Merismopedia
Unicellular or Colonial	Budding Multiple Fission = splitting in more than two parts	Chamaesiphonales	Chamaesiphon Pleurocapsales Dermocarpa Xenococcus Pleurocapsa
Filamentous	Trichome, which is a chain of cells	Nostocales	**Oscillatoria** Microcoleus Lyngbya Phormidium Schizothrix Spirulina Plectonema
Filamentous heterocystous	Trichome fragmentation = splitting of the chain of cells	Nostocaceae Rivulariaceae Scytonemataceae	Anabaena Nostoc Cylindrospernum Calothrix Rivularia Scytonema
Branched filamentous	Trichome fragmentation	Stigonematales	Westiella Fisherella Stigonema Chlorogloeopsis

2.2.6 Cyanobacterial toxins

Certain species of cyanobacterial produce naturally poisonous substances called toxins. These toxins fall into various categories. Some are known to attack the liver (hepatotoxins) or the nervous system (neurotoxins); others simply irritate the skin. These toxins are usually released into water when the cells rupture or die. Health scientists are more concerned about hepatotoxins than neurotoxins, because neurotoxins are not considered as widespread as hepatotoxins in water reservoir (Graham, et al., 2000, Amit and Datta, 2005). Toxins produced by cyanobacteria are widely distributed about 80 specific molecular structures have been discovered in just the last 15 years. These toxins are divided into neurotoxins (about 20), which are generally alkaloids, hepatotoxins, which are cyclic peptides often-called microcystins (about 60), and lipopolysaccharide endotoxins (unknown number) that are compounds of fats and sugars. The neurotoxins and microcystins have the highest toxicities, are of most concern to EPA and local water authorities (EPA, 2005).
the diversity of highly toxic substances that can be produced by growing cyanobacteria on a body of nutrient-rich water.(EPA, 2000).

Some species of cyanobacteria produce toxins that affect animals and humans. People may be exposed to cyanobacterial toxins by drinking or bathing in contaminated water. The most frequent and serious health effects are caused by drinking water containing the cyanobacterial toxins or by ingestion during recreational water contact (Graham,et al.,2000).

Disease caused by cyanobacterial toxins varies according to the type of toxin and the type or water-related exposure (drinking, skin contact, etc.). The disease symptoms including skin irritation, stomach cramps, vomiting, nausea, diarrhea, fever, sore throat, headache, muscle and joint pain, blisters of the mouth and liver damage. Swimmers in water containing cyanobacterial toxins may suffer allergic reactions, such as asthma, eye irritation, rashes, and blisters around the mouth and nose. Animals, birds, and fish can also be poisoned by high levels of toxin-producing cyanobacteria (Charles and Stoltenow, 1997).

Some species of cyanobacteria such as Microcystis, Anabaena, Oscillatoria, Nodularia, Nostoc, Cylindrospermopsis and Umezakia. Produced hepatotoxins that also called microcyctin while some strains of Aphanizomenon and Oscillatoria produced neurotoxins

which also called anatoxins. Cyanobacteria from the species Cylindroapermopsis raciborski may also produce toxic alkaloids, causing gastrointestinal symptoms or kidney disease in humans (Haok, et al.,1995). Contact with water contaminated by cyanobacteria toxins is very dangerous on human health especially for children, so this contact should be avoided (WHO,2001).

2.2.7 Water contamination by cyanobacteria toxins

Some 50-75 % of cyanobacterial blooms generate toxins, and some experts believe that all cyanobacterial blooms should be regarded as toxic until disproved (Graham,et al.,2000, Obsborn, et al., 2001). Because no one can tell if this bloom is toxic or not, water samples have to be analyzed in a laboratory to detect if this water body is safe. Microcystin can persist in freshwaters for two or more weeks before being regarded by bacteria. Significant increases in the rate of liver cancer in the Chinese village has been correlated with contamination of surface drinking water supplies by microcystin (Hoak,et al.,1995). However, sometimes it is hard to tell if the drinking water has been contaminated unless confirmed by laboratory tests specifically for measuring cyanobacteria toxins levels such as ELIZA (Enzyme Linked Immunosorbent Assay).The ELIZA procedure is useful in determining how much microcyctin founds in water(Graham,et al.,2000). Municipal water treatment plants remove variable amounts (sometimes up to 98%) of microcyctins from water. Although ozonation, treatment with activated charcoal, and chlorination can remove microcyctin, in some caces, as much as 20% may escape from the treatment process (Hoak,et al.,1995).

2.3 Oscillatoria species

Oscillatoria is a genus of filamentous cyanobacteria which is named for the oscillation in its movement (Oscillator means something that swings).The name reflects this organisms ability to rotate, or oscillate. It is commonly found in high nutrient waters, and is mainly blue-green or brown-green. Oscillatoria is an organism that reproduces by fragmentation (Arieli, et al.,1994). Oscillatoria forms long unbranched filaments of cells which can break into fragments called hormongia. The hormogonia can grow into a new, longer filament. Breaks in the filament usually occur where dead cells are present.Most tolerant of organic pollutants (Hoak,et al.,1995).

2.3.1 Taxonomic Description of Oscillatoria

Individual cells are disk-shaped cells that are typically wider than long are attached end to end to form filaments (Graham,et al.,2000).The end cell may be rounded or distinctive in ways that are used to define some of the species. Breakage of filaments between separation disks releases shorter homogonia during vegetative reproduction. Filaments may sometimes adjoin in parallel to form thin films, but a readily identifiable sheath is not present (Hoak,et al.,1995).

2.3.2 Oscillatoria Ecology:

Oscillatoria are often the dominant cyanobacteria in eutrophic lakes, ponds and streams. Typically forms mats or films across the water surface (Graham,et al.,2000).

Optimal temperatures for growth ranges from $15C^{o}$ -$35C^{o}$. Slow growth rates and tolerance to desiccation. Freeze-thaw cycles, and high solar irradiation also contribute to the survival of Oscillatoria in aquatic ecosystems (Hoak,et al.,1995).

Oscillatoria has been found in wide variety of environments, including hot springs, marine habitats, and lakes, tropical, and polar regions, as well as moist terrestrial substrates (Graham,et al.,2000).

2.4 Literature Review:

" The use of Marine invertebrates as indicator of water quality". Microorganisms can act as indicators of water quality and of environmental problems in aquatic ecosystems. Algae for example grow quickly and are sensitive to changing environmental conditions. They are often among the first organisms to respond to changes. They offer a signal of the biological condition in a watershed. Using bio indicators as an early warning of pollution or degradation in an ecosystem can help sustain critical resources (**Fischer,2002)**.

"Use of cyanobacteria to assess water quality in running waters**"** The correlation between Oscillatoria abundance and the nutrient content reflected that this type of cyanobacteria was predominant because of the high concentration of nutrient (Douterelo**, et al., 2004**).

"Use of Fourier Transform Infrared Spectroscopy for Typing of Candida albicans Strains isolated in intensive care units**"** FTIR spectroscopy is increasingly being used for the identification of microorganisms at the species level. It can identify each type of microorganism through its special chemical functional groups series(**Sandt , et al. ,2002).**

"Structure and dynamics of membrane proteins as studied by infrared spectroscopy**"** Each type of microorganism cell membrane contain a different set of proteins that have different

chemical composition which will specialized it from other type of microorganisms. It is a fingerprint for this chemical structure and so to the microorganism. These sets is easily detected by FTIR because it is a very successful tool for detecting different proteins types especially membrane proteins (**Jose, et al,1999;Albert et al.,2004).**

"Colorimetric Immuno-Protein Phosphatase Inhibition Assay for Specific Detection of Microcystins and Nodularins of Cyanobacteria". The health risks posed by cyanobacterial toxins and the increasing anthropogenic eutrophication of potable and recreational waters have increased the need for rapid, sensitive method like FTIR to determine the presence of this type of cyanobacteria in water(**James, et al.,2001)**.

"Assessing Potential Health Risks from Microcystin Toxins in Blue-Green Algae Dietary Supplements". Exposure to Oscillatoria toxins through the ingestion of contaminated water or recreational contact is receiving increasing attention around the world as a public health concern . Recently the World Health Organization, have made recommendations regarding safe cyanobacteria toxin levels in drinking water and surface waters used for recreation **(Duncan, et al.,2000)**.

"Highly sensitive detection of proteins on membranes with near infrared fluorescence" Common visible fluorophores cannot be used effectively for direct protein detection because of high background fluorescence of the membranes in visible range. Near infrared fluorophors (670-1100 nm) have a distinct advantage over visible fluorophores. Very low background fluorescence at longer wavelengths provides an excellent signal to noise ratio and then protein chemical functional groups series is easily detected using FTIR (**Amy etal.,1998)**.

" Molecular biology of the cell ". Cell membrane are asymmetrical structures: the lipid and proteins compositions of the outside and inside faces differ from one to another in way that reflect the different functions performed at the two surfaces of the membrane(**Alberts et al.,2001).**

"Fourier Transform Infrared spectroscopy of Pediastrum duplex: characterization of micro-population isolated from a eutrophic lake**"** FTIR is excellent method not only for qualitative determination of microorganisms found in water but also for quantitative analysis of microorganisms which means that it can be used for exact determination of the water quality and its uses. FTIR has been widely used to provide information on a range of vibrationally

active functional groups (including O-H, N-H, =C-H, -CH_2 and P=O in microorganisms. FTIR spectroscopy has the spatial resolution to analyze single cells and microorganisms within mixed water samples. Phytoplankton and lake water samples had been collected from Hollingworth Lake (Rochdale) and Rostherne Mere (Knutsford) during the summer bloom period of 2001. All samples were followed by air-drying under sterile laminar flow at room temperature(**Sigee, et al.,2001)** . Results were as follow:

Cultured cells. Single cultured cells of Scenedesmus generated FTIR spectra with 12 clear peaks (Figure 4). Contribution from reactive molecular groups in water, lipids, proteins, starch and nucleic acids). Table 3 shows tentative assignment of these peaks.

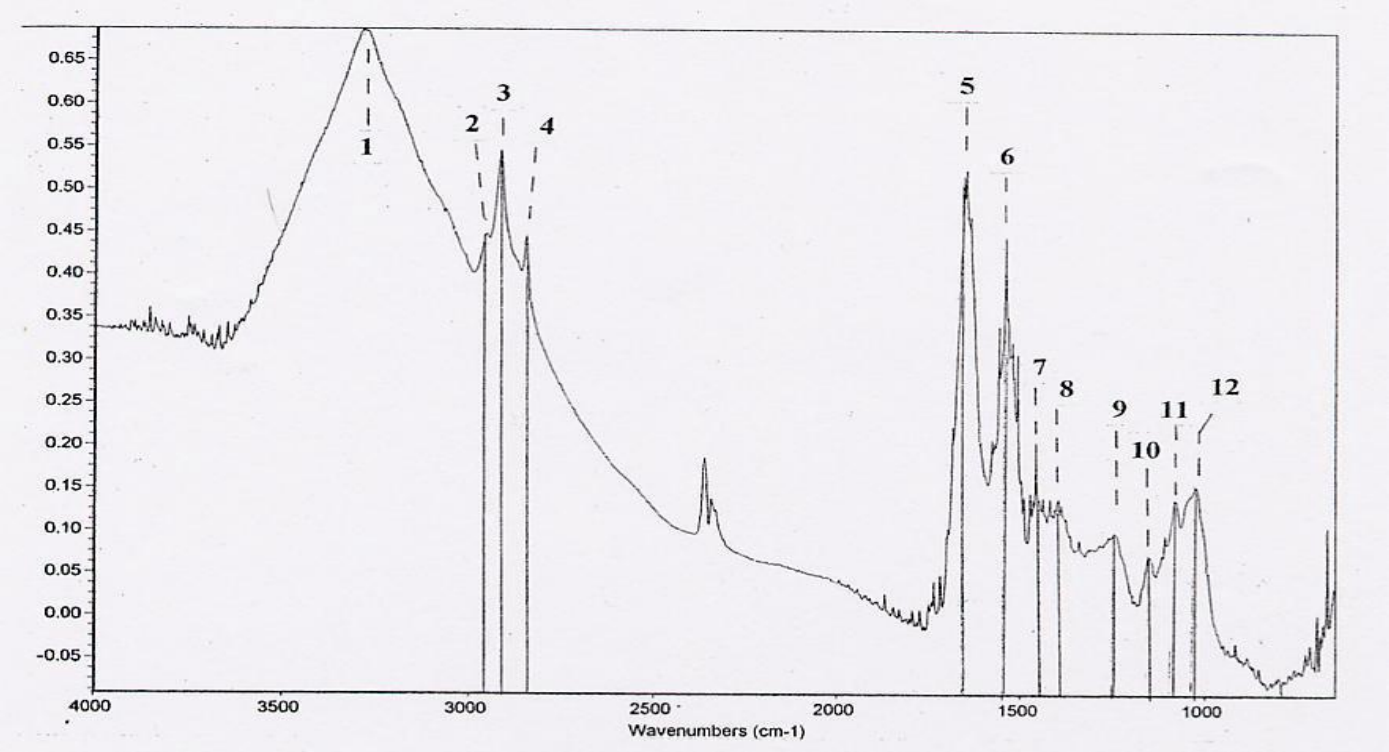

Figure 4: FTIR spectrum from air-dried cultured cell of Scenedesmus(Sigee,2001).

Table 3:Tentative assignment of peaks found in FTIR spectra of air-dried colonies of Scenedesmus (Sigee,2001).

Peak number	Peak wavenumber (cm^{-1})	Wavenumber range (cm^{-1})	Tentative assignment of bands
1	3300	3646-3026	WATER (1) ν(O-H) stretching PROTEIN (2,6,7) ν(N-H) stretching (amide A)
2 ------- 3	2955 ---------- 2909	2943-2902	LIPID (3,6,7) $\nu_{as}(CH_2)$ stretching of methylene
4	2840	2864-2837	LIPID (3,6,7) $\nu_{s}(CH_2)$ stretching of methylene
5	1660	1709-1587	PROTEIN (2,4,6) amide 1
6	1547	1577-1481	PROTEIN (4,6) amide II band DNA (5) Double bond vibrations of bases
8	1454	1477-1421	LIPID (3,7) $\delta_{as}(CH_2)$ bending of methyl
9	1250	1294-1194	NUCLEIC ACID (5) ν_{as}(>P=O) stretching of phosphodiesters
10	1159	1196-1136	STARCH (1) ν(C-O) stretching and complex sugar ring modes
11 ------- 12	1094 ---------- 1038	1136-980	STARCH (1) ν(C-O) stretching and complex sugar ring modes NUCLEIC ACID (5) ν_{s}(>P=O) stretching of phosphodiesters

2.5 Sampling Sites:

Jericho is a green oasis in the Jordan Valley which lies 7 km west of the River Jordan, 10 Km north of the Dead Sea and 30 Km east of Jerusalem. It lies 370 meters below sea level and thus it is considered to be the lowest city in the world.

The average temperature in January is 8.5 degrees and the lowest average annual temperature is 19 degrees. The average annual temperature is 23.5 degrees and the highest average annual temperature is 38 degrees. The average annual amount of rainfall is 120-250 millimeters, and the average annual humidity is 52% .The amount of rainfall in the Jericho area is less than that of the surrounding mountains and the coastal regions, thus Jericho area relies entirely for drinking and irrigation on subterranean wells and springs such as the Ein Al-Sultan spring. The source of this water is situated in the distant mountains. Ein Al-Sultan spring in considered to be the main source for agriculture. It has an output of 680 cubic meters per hour, and a salinity of 600 fractions in one million. It provides a steady output throughout the year. It is used equally for drinking water and for irrigating. Jericho is considered to be an important area for agriculture. It is famous for its citrus fruits, dates, bananas, flowers and winter vegetables. The area within the municipality limits is about 45 square kilometers, and the population of the city of Jericho alone is 17,000. If the population of the surrounding villages and refugee camps are included the number goes up to 25,000 inhabitants. Jericho is also one of the lowest cities in the world, about 800 feet (244 m) below sea level.

Bethlehem: The town of Bethlehem is situated on a prominent limestone ridge in the Hill Country about five miles south of Jerusalem. At an elevation of 2,500 feet, According to the Bethlehem Municipality, the city has a total area of 6.0 $\underline{km^2}$.

Population: 21,947 inhibitants .Topography: 32 degrees north of the equator, 2,500 feet (760 m) above sea level .Climate: winter from November to March, coldest in January with high of 13 to low of 1 degree Celsius; summer from June to September, warmest in August with high of 27 to low of 17 degrees Celsius.

Bethlehem has a commanding view of the surrounding terrain. Tourism and agriculture drove the economy of Bethlehem. The fertile hill country surrounding the town supported cereal

crops, vineyards and olive orchards, as well as abundant grazing land for sheep. Figure 5 shows the location of Jericho and Bethlehem region in the West Bank.

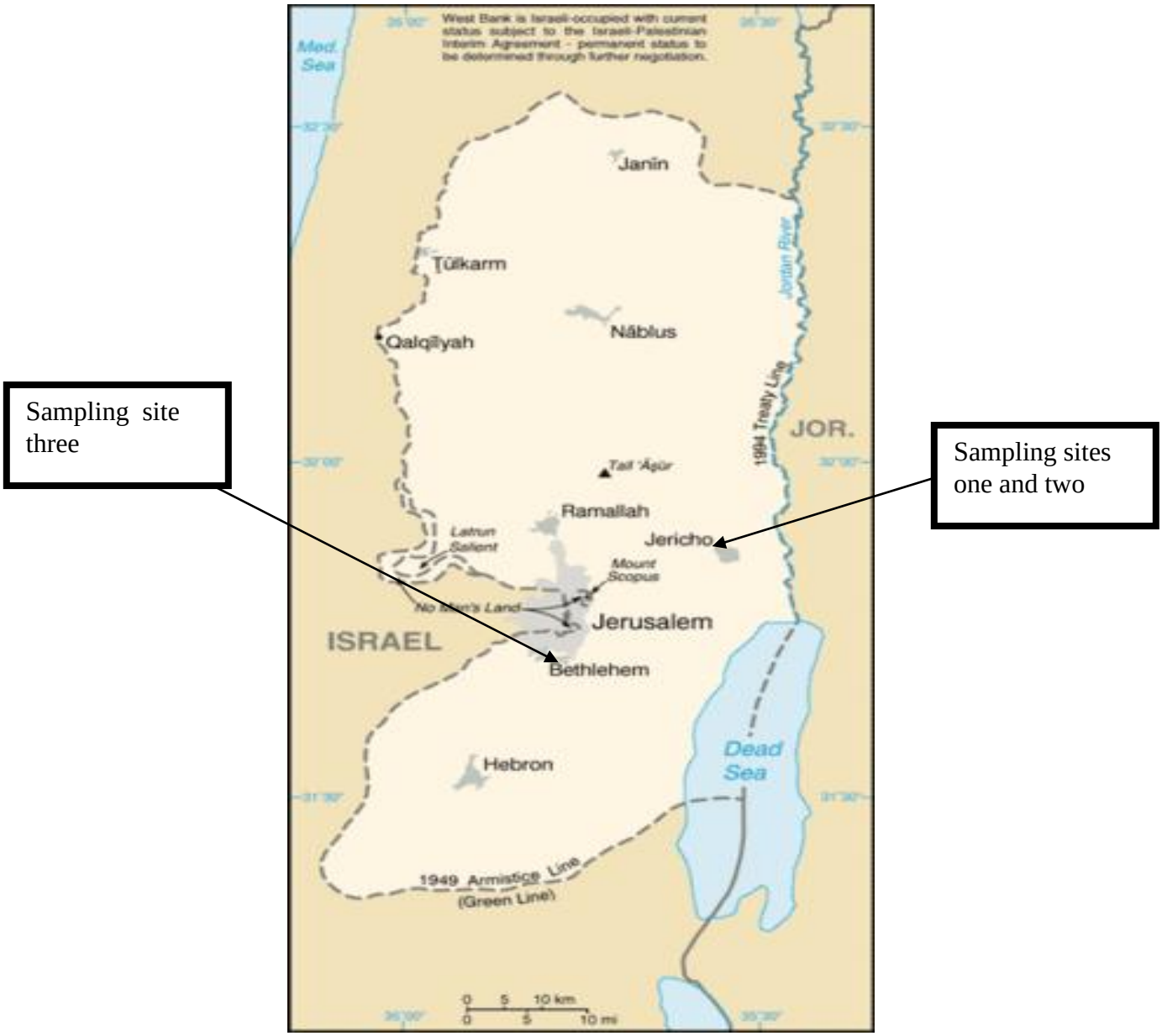

Figure 5: Jericho, Bethlehem, West Bank\Palestine(Sampling Sites).

Three sampling sites were studied:

Site I : Jericho aquaculture (Alhusseini pond (15m length X6m wedthX3m depth) concrete pond, fed directly from Wadi-Alquilt).

Site II: an Agriculture pond. (Ein sultan pond (4m lengthx4m wedthx3m depth) concrete ponds feeds directly from Ein sultan and used mainly for irrigation

SiteIII: Sulaiman ponds Located 3 km southwest of Bethlehem

They comprise three neighboring pools that were arranged in such a manner that the first pool poured into the second, and the second into the third, and from which canals branched out. The first pool is 116 m long and approximately 72 m wide. Its depth ranges from between 6 m and 12 m, and its capacity was 85,000 cu m. The middle pool is 129 m long and 76 m wide. It is 12 m deep and its capacity was approximately 90,000 cu m. The lower, third pool is the biggest of the three at 177 m long and 86 m at its widest point. It is around 15 m deep and had the greatest capacity of around 113,000 cu m.

2.6 Statement of the problem

Damage to the overall condition of aquatic resources in the West Bank due to anthropogenic influence, is becoming a serious problem, whereby an increase in pollutant levels is associated with a decrease in quality of water resources. Knowledge of the state of water quality in aquatic ecosystems and the changes produced by human activities is the first step towards establishing an efficient water management system, as is essential for the preservation of these ecosystems. Many methods are currently used for water quality determination based on bioindicators. Most of these methods are very expensive, time consuming or may be specific for certain types of microorganisms. The presence of different kinds of microorganisms in watershed is a direct indicator of the water quality, types of pollutants and also its sources. As an example the presence of E.coli bacteria is a direct indication of fecal contamination while the presence of Oscillatoria as an example of cyanobacteria is an indication of high nutrient level.

Oscillatoria presence in water not only shows bad water quality but also it produces serious types of toxins: neurotoxins and hepatotoxins. Neurotoxins are very dangerous to livestock and human they directly attack the nervous system and block the signals transmission from neuron to neuron and neuron to muscle while hepatotoxins cause bleeding in the liver.

Exposure to Oscillatoria toxins through ingestion of contaminated water or recreational contact is receiving increasing attention around the world as a public health concern. Recently the World Health Organization ,have made recommendations regarding safe cyanobacteria toxin levels in drinking water and surface waters used for recreation. (Duncan et al.,2000).

The health risks posed by cyanobacterial toxins and the increasing anthropogenic eutrophication of potable and recreational waters have increased the need for rapid, sensitive, inexpensive method like the FTIR method proposed in this investigation to determine the presence of this type of cyanobacteria, and other different types of microorganisms found in water which was reflect the water quality.

2.7 Hypotheses

In order to conduct this research, the following hypotheses were developed. The development of these hypotheses were abstracted from literature review, these were:

1. Microorganisms can serve as bioindicators for water quality because each microorganism prefer its suitable condition for growth and survival, the presence of certain types of microorganisms mainly algae will leads to exact determination of water components.
2. Oscillatoria as a cyanobacteria species is directly proportional to high nutrient contents level in the water body, as the water body becomes more eutrophic the Oscillatoria presence will increase showing bad water quality.
3. Early detection of water quality will lead to preserve the water resources and avoid water scarcity.
4. FTIR is a suitable technique used for the determination of types and amounts of different microorganisms founded in water because it can differentiate between each type of microorganism.
5. FTIR is preferred for the water quality determination because it is a rapid and a very cheap method can be easily used to run each water sample.

6. Identification of low amount of microorganisms founded in water using microscope is very difficult while FTIR can detect very small amount of microorganisms.
7. Each microorganism will have special fingerprint depends on its special chemical functional groups series in its membrane proteins , this fingerprint will be easily detected if the water sample containing it is analyzed using FTIR .

2.8 Theoretical Frame work

FTIR has been widely used to provide information on a range of vibrationally active functional groups (including O-H, N-H, =C-H,-CH_2 and P=O in microorganisms.

Although the technique has been largely used with microorganism complexes such as nucleic acids, proteins mainly cell membrane proteins because they are specific for each microorganism (Sigee, 2001).

2.8.1 Structure of microorganism cell membrane

Cell membranes or, the plasma membrane encloses the cell, defines its boundaries, and maintains the essential differences between the cytosol and extra cellular environment (Jose, et al., 1999).

All biological membranes have a common general chemical structure: each is a very thin film of lipid and protein molecules, held together mainly by no covalent interactions (Amy, et al., 1998). Cell membranes are dynamic, fluid structures, and most of their molecules are able to move about in the plane of the membrane. The lipid molecules are arranged as a continuous double layer about 5 nm thick, this lipid provides the basic structure of the membrane and serves as a relatively impermeable barrier to the passage of most water-soluble molecules (Arrondo, et al., 1999). Protein molecules dissolved in the lipid bilayer mediate most of the other functions of the membrane, transporting specific molecules across it, or catalyzing membrane associated reaction, such as ATP synthesis (Figure 6).

In the plasma membrane some proteins serve as structural links that connect the membrane to the cytoskeleton and to either the extra cellular matrix or an adjacent cell, while others serve as receptors to detect and transducer chemical signals in the cells environment (Keeton, 1983).

Cell membrane are asymmetrical structures: the lipid and proteins compositions of the outside and inside faces differ from one to another in way that reflect the different functions performed at the two surfaces of the membrane (Alberts et al.,2001).

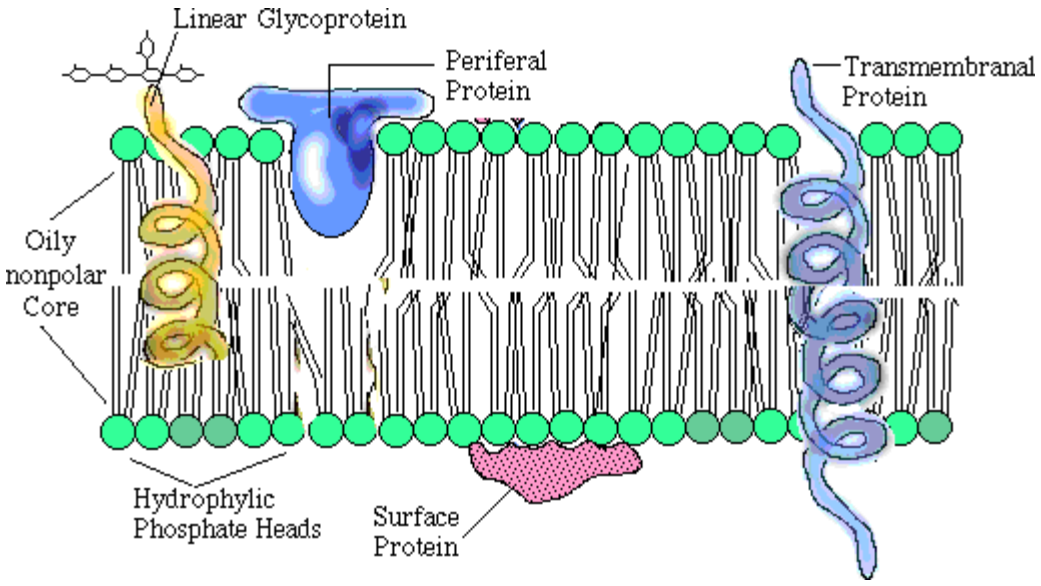

Figure 6: Microorganisms cell membrane structure (Keeton,1983).

2.8.2 The lipid bilayers:

Biological membranes consists of continuous double layer of lipids molecules in which various membrane proteins are embedded. This lipid bilayer is fluid, with individual lipid molecules able to diffuse rapidly within their own monolayer (Amy, et al., 1998). Membrane lipid molecules are amphipathic, and some of them (the phospholipids) assemble spontaneously into bilayers when placed in water; the bilayers formed sealed compartments that reseal if torn. There are three major classes of membrane lipid molecules: phospholipids, cholesterol, and glycolipids, the lipid compositions of the inner and outer monolayer are different, reflecting the different functions of the two faces of a cell membranes (Alberts, et al.,2001).

2.8.3 Membrane proteins:

Whereas the lipid bilayer determines the basic structure of biological membranes, proteins are responsible for most membrane functions, serving as specific receptors, enzymes, transport proteins (Keeton,1983).

Many membrane proteins extend across the lipid bilayer in some of these transmembrane proteins the polypeptide chain crosses the bilayer as a single alpha helix in others, responsible for the transmembrane transport of ions and other small water-soluble molecules, the

polypeptide chain crosses the bilayer multiple times, either as a series of alpha helices or a beta sheet in the form of a closed barrel (Arrondo, et al.,1999) .

Other membrane associated proteins do not span the bilayer but instead are attached to one or the other side of the membrane .many of these is bound by noncovalent interactions with transmembrane proteins, but others are bound by covalently attached lipid groups. Like lipid molecules in the bilayer, many membrane proteins are able to diffuse rapidly in the plane of the membrane (Keeton,1983). On the other hand, cells have ways of immobilizing specific membrane proteins and of confining both membrane protein and lipid molecules to particular domains in a continuous lipid bilayer.(each type of cell membrane contains a different set of proteins, reflecting the specialized functions of particular membrane(Albert et al. ,2004)

In the plasma membrane of all eukaryotic cells most of the proteins exposed on the cell surface and some of the lipid molecules in the outer lipid monolayer have oligosaccharide chains covalently attached to them. Some plasma membranes also contain integral proteoglycan molecules with surface-exposed polysaccharide chains, this sugar coating helps to protect the cell surface from mechanical and chemical damage, and some of the oligosaccharide chains are recognized by cell-surface carbohydrate-binding proteins (lectins)that mediate specific, transient, cell-cell adhesion events(Alberts, et al.,2001).

2.8.4 Advantages of FTIR spectroscopy

FT-IR spectroscopy produces conventional spectrum and it has several important advantages:

• The most important advantage of FT-IR spectroscopy for biological studies is that spectra of almost any biological material can be obtained in a wide variety of environments.

• The amount of sample required is relatively small.

• FT-IR method is a rapid and sensitive technique with sampling techniques that are easy to use (Kemp, 1987).

• Since a computer is already used to obtain the Fourier transform, it is easy to perform many scans to improve the signal-to-noise ratio.

• FTIR is inexpensive compared to the cost of X-Ray diffraction, NMR, equipment and the operation of the equipment is simple. Interpretation of the spectra is not particularly difficult and can be learned easily.

• Digital substraction (that is, point-by-point substraction of the separate spectra by a computer) can also be used to produce good difference spectra. This method has great advantages in obtaining infrared spectra in aqueous solutions.

• The FT-IR has advantage in terms of spectral regions which originate from molecular vibrations and different molecular moieties. For instance "head group" and "hydrocarbon tails" have spectral regions for membranes (Kemp, 1987).

• There is no light scattering or fluorescent effects.

• Kinetic and time-resolved studies are possible (Sherman, 1995).

2.8.5 Infrared spectroscopy as a tool for the microorganisms studies

Last years have new sensitive, rapid, and increasingly precise physical techniques for microbiological analysis. These new techniques range from various spectroscopic techniques such as molecular spectroscopy (including FTIR and mass spectroscopy) to the different separation techniques like gas chromatography (GC) and high-performance liquid chromatography (HPLC) (Sigee, et al.,2001) . In 1911 W.W.Coblentz was probably the first scientist to suggest that biological materials can profitably be analyzed by means of infrared (IR) spectroscopy (Arrondo, et al., 1999). Already in the 1950s and 1960s spectroscopists have demonstrated the suitability to identify bacteria by IR spectroscopy (Sandt, et al., 2002). Unfortunately, due to the lack of efficient computers and the weak instrumental specifications at that time, reports on bacterial characterizations by IR spectroscopy became less frequent in the 1960s and less in the 1970s(Sigee, et al.,2001). It is the development of modern interferometric IR spectroscopy and the availability of low-cost computers that contributed greatly to revival of IR spectroscopy as a means for characterizing intact microorganisms in the last years. Because FTIR spectroscopy is a nondestructive technique and allows the rapid and simultaneous characterization of complex materials like microorganisms and studies the ability to differentiate microorganisms from various sero groups it is widely used in microorganisms determination (Arrondo, et al., 1999).

2.8.6 Advantages of FTIR spectroscopy for the identification of microorganisms

Traditional methods such as polymerase chain reaction (PCR) method (based on 16S rDNA sequencing and DNA-DNA hybridization), PCR-enzyme-linked immunosorbent assay (ELISA) flow cytometric technique), fiber-based multiplex PCR assay and using selective and

differential media used for the identification of microorganisms can often be extremely tedious, offering results that may take time and occasions not prove totally conclusive. (Arrondo, et al., 1999). FTIR technique can be used as an analytical tool in various fields (clinical, environmental, food microbiology) for the very rapid classification, differentiation of diverse microorganism's species and strains (Sigee, et al., 2001).

FTIR Spectra reflect the overall molecular composition of a sample, since different organisms differ in overall molecular composition, their FTIR spectra will also be different. The spectra can serve as spectroscopic fingerprints that enable highly accurate identification of microorganisms (Sandt, et al., 2002).

2.9 Objectives of this study

Traditional methods used for the identification of various microorganisms
Are based on morphological characteristics, molecule structure etc. However, all of these methods can often be extremely tedious, offering results that may take time and on occasions not prove totally conclusive.

FTIR spectroscopy is a valuable technique due to its high sensitivity in
Detecting changes in the functional groups belonging to the components
Of cyanobacteria, such as lipids and proteins. This technique, which requires neither reagent nor sample preparation, is non-destructive, and highly selective because
Of its ability to be a spectral fingerprint for molecular components.

This study aims to:

1. To investigate the optamization of FTIR spectroscopy as an method for water quality monitoring by the determination of different kinds of microorganisms in the aquatic ecosystem in the West Bank region-Jericho.
2. To check the suitability of FTIR spectroscopy for the quantitative study of these microorganisms.

Chapter 3

Methodology

3.1 Collection of samples

The centers of three ponds with different characteristics were studied: Alhusseini pond (sampling site I), Ein sultan ponds (sampling site II) both are located in Jericho region and Sulaiman ponds (sampling site III), which are located in Bethlehem region. Sampling sites were selected in order to include different kinds of microorganisms due to differences in environmental condition in both regions. Sampling site I was monthly sampled throughout one year (2006-2007), while only one water sample was collected throughout March 2007 from sampling sites two and three. Table 4 show sampling sites, date of collection, volume filtrated from each samples.

Table 4: Volume filtrated from each water samples collected from sampling sites I,II and III.

Sample No.	Sample Date	Sampling site	Volume of filtered water (mL)
1	22/1/2006	Site I	1000
2	24/2/2006	Site I	1000
3	19/3/2006	Site I	1000
4	24/4/2006	Site I	500
5	22/5/2006	Site I	500
6	26/6/2006	Site I	300
7	10/7/2006	Site I	100
8	24/8/2006	Site I	100
9	23/9/2006	Site I	100
10	3/10/2006	Site I	300
11	27/11/2006	Site I	500
12	20/12/2006	Site I	1000
13	23/1/2007	Site I	1000
14	8/3/2007	Site II	500
15	14/3/2007	Site III	500

3.2 Effects of nutrients

Water samples for chemical and biological analysis were collected in polyethylene bottles. Nutrient chemical concentration ($No_{3,}$ Po_4) were determined in Environmental laboratory in Al-Quds university using colorimetric methods, Water samples were rapidly processed within few hours of collection from the aquatic resources to minimize cell deterioration and changes in chemical composition (EPA,1983). Table 5 describes the indicators used in the regular water quality monitoring.

Table 5: Nutrient level as indicator for water monitoring (EPA, 2007).

Category	Indicator	Explanation
Nutrients	Nitrogen Organic Nitrate plus nitrite Ammonia Total	The nutrients nitrogen and phosphorus are essential for plant growth. High concentrations indicate potential for excessive weed and algal growth.
	Phosphorus Filterable reactive Total	Total nutrients are made up of a dissolved component (e.g. nitrate plus nitrite, ammonia and filterable reactive phosphorus) and an organic component, which is bound to carbon (e.g. organic nitrogen). Nutrients in the dissolved state can be readily used by plants.

3.3 Qualitative Test:

3.3.1 Microscope Screening

Wet mount preparation was done for all water samples to be screened for microorganisms using (Leica) compound microscope. Preparations for microscopy involved with no treatment. The general procedures would be expected to result in minimal alteration in the chemical composition of these types of microorganisms and the spectra obtained were considered representative of these microorganisms found in water sample.

3.3.2 Oscillatoria standard preparation

A standard of Oscillatoria was prepared by isolating one filament under microscope .The filament was returned to the same filtered water sample, which contains no microorganisms. Growth was enhanced using Sunlight. The closed vial was incubated at room temperature for

about one week. Filter paper with small pores (small pores 0.45 um), was used in order to eliminate all microorganisms that were found in the water sample because their size is larger than the filter paper pores (Keeton, 1983). The water sample was screened under the microscope before and after Oscillatoria filament was incubated when water sample in the closed bottle was screened again using the microscope only Oscillatoria was presented, the sample was filtered and prepared for FTIR analysis.

3.3.3 Negative control preparation

About 500ml of water sample number 9 which was collected at 23/9/2006 from sampling site I and contains Oscillatoria when it was screened by the compound microscope was autoclaved for about 15 minutes in order to kill Oscillatoria. A negative control was needed in our work to be sure that the fingerprint which was obtained when the water sample was analyzed using FTIR was for Oscillatoria and not for any other pollutants.

3.3.4 FT-IR procedure

A Fourier Transform Infrared Spectrometer collects and processes infrared wavelength absorbance or transmissions into spectra. These spectra are created when a molecule converts infrared radiation into molecular vibrations. These vibrational movements create bands in a spectrum that occur at specific wave number (cm^{-1}) (Dudley, et al., 1987).

Each wavelength is then further dependent on a number of other issues that can be used to help identify which types of bonds are present in the test specimen (Sherman, 1994).

Depending on the types of chemical bonds present in the specimen, the radial light will be absorbed, transmitted, or reflected at the various wavelengths. From the spectrum produced by the sample, information about the specific bonding present is obtained from the location of group frequency peaks. (Kemp, 1987). Dry air has been continuously purged into the spectrometer to get rid of water vapor, which disturbs the signals. Water and carbon dioxide molecules in the air affect the IR spectrum. To overcome this problem the spectrum of air was recorded as background and subtracted automatically by using appropriate software (Omnic software) (Sigee, et al., 2001).

FT-IR spectra of cyanobacteria samples were recorded in the 4000-400 cm^{-1} region at room temperature. Atmospheric vapor was automatically subtracted from the sample spectrum via Omnic software.

3.4 The Effect of overlapping on Oscillatoria fingerprint

3.4.1 Escherichia coli (E.coli)

The coli form group consists of several genera that belong to the Enterobacteriaceae. The historical definition was based on the method used for detection (lactose fermentation) rather than scientific principles of systematic bacteriology. Accordingly, organisms in this group are defined as: all aerobic and facultative anaerobic, gram-negative, non spore-forming, rod-shaped bacteria that ferment lactose with gas and acid formation within 48h at 35C^{o} (Keeton, 1983). Members of this group may come from a variety of sources, but public health officials are interested in those that may have their origin in the intestinal tract of warm blooded animals and pass through with feces (fecal coli forms). The rationale is that if fecal coli forms are present, then there is a certain risk that one or more parasitic organisms may also be present. The specific presence of Escherichia coli (E. coli), which is the microbial indicator of water quality (Brock, et al., 2000).

Isolation of E.coli

E.coli as coli form bacteria was cultured on macconcy agar in biology laboratory at Al Quds University. Macconcy agar is a selective media for this type of bacteria (Keeton.1983); a pure E.coli colony was isolated, lyophilized and then analyzed by FTIR in order to be compared by Oscillatoria fingerprint.

3.4.2 The effect of filter paper porosity

In order to study the probability of overlapping of the Oscillatoria fingerprint with other types of microorganisms that are found in the same water sample, water sample No.14 which was collected from site II at 8/3/2007 was filtered using two different whatman filter paper, the first one with 2.7um pores size was used in order to eliminate only Oscillatoria filaments because they has size about 50 µm (Graham, et al., 2000) which is larger than the filter paper pores. All other microorganisms will go down through the pores of the filter paper because they are smaller in size than the filter paper pores (Bacteria and small invertebrates) (Keeton, 1983). The second filter paper with 0.45µm pores size was used to filter the same water

sample. All microorganisms founded in the water sample including Oscillatoria, bacteria and small invertebrate were eliminated on the filter paper because all of them are larger in size than the filter paper pores size. The two types of filter papers with microorganisms on were lyophilized and analyzed using FTIR spectrometer.

3.5 The distribution of Oscillatoria in West Bank

The climate of Jericho is arid, which is hot in summer and warm in winter, January is the coldest month with an average of 19C^o and August is the hottest month with an average of 38C^o while Bethlehem climate which is cold in winter, with high of 13 to low of 1 C^o in January and, moderate in summer with high of 27 to low of 17 C^o in August. Jericho climate conditions are suitable for Oscillatoria which has optimal temperatures for growth ranges from 15C^o-35C^o (Hoak, et al., 1995) rather than Bethlehem region.

Two water samples, sample No.14 which was collected from sampling site II at 8/3/2007 and sample No.15 which was collected from sampling site III at 14/3/2007 were analyzed using FTIR in order to study the distribution of Oscillatoria in the West Bank by comparing the fingerprint of each water sample with Oscillatoria standard fingerprint.

3.6 Quantitative Test

Water sample No.8 which was collected from sampling site I at 30/8/2006. This water sample was firstly screened by the compound microscope. Results of microscopic screening showed Oscillatoria filaments. The water sample was then exposed to sunlight in order to enhance Oscillatoria growth and proliferation for about one week from 30/8/2006-6/9/2006. About 100ml from this sample were filtered daily using Whatman filter paper with 2.7um pores to isolate only Oscillatoria filaments which have larger size than the pores of the filter paper .All these water samples were also analyzed daily by FTIR for one week. Table 6 shows the quantitative test procedure:

Table 6: Quantitative Test.

Sample No.	No. of exposure days	Volume of filtered water (mL)	Microscopic results(Oscillatoria filament number)	FTIR result (Transmittance %)
8	1 (30/8/2006)	100		
	2 (31/8/2006)	100		
	3 (1/9/2006)	100		
	4 (2/9/2006)	100		
	5 (3/9/2006)	100		
	6 (4/9/2006)	100		
	7 (5/9/2006)	100		

3.7 Laboratory Work

All water samples were analyzed following the same procedure in biology lab at Al-Quds University to be analyzed biologically by:

1- **Microscope**: Firstly about 1ml of each water sample which were collected from the three different sampling sites were prepared in the microscopic slides, it was covered by cover slip (22mmx22mm) then screened by the microscope to investigate what types of different microorganisms were presented in each water sample.

2- **Filtration of samples:** All water samples were filtered by filtration set using Whatman filter papers with 2.7um pores to separate Oscillatoria on . Only Oscillatoria filament was isolated on this filter paper types because they Oscillatoria filaments are larger than the filter paper pores (Oscillatoria filament about 50um in size (Graham, et al.,2000)).All other microorganisms which smaller than the filter paper pores like bacteria and small invertebrates will go down with water . A pump was used to accelerate the filtration process.

3- **Lyophilizing:** The filter papers with the filtered microorganism were placed in small beaker. It was closed by Kim wipes. The beaker was placed in the lypholizer chamber. The lypholizer chamber was then connected to (LABCONCO) lypholizer to be dried freeze at temperature less than $-40C^{o}$ and pressure equal 0.06mbar.

4- **Sample preparation for FTIR studies** :

Dry cyanobacteria samples were grinded using mortar and pestle to produce powder mixture of filtrate powder, about 0.4 mg of cyanobacteria was mixed with 400 mg potassium bromide (at a ratio of 1/100). The KBr is most commonly used alkali halide disk which is used as a beam condensing system (Sherman, 1994). Transparent disks were prepared by establishing pressure of (9000 pound per square inch (psi)) in compressor (Hidropn◌mak) for about 6 minutes (Sigee, et al., 2001).

Infrared analysis was carried out at Biology laboratory, Al-Quds University using Nicolet AVATAR 370 FTIR spectrometer. All manipulation of spectra was carried out using Nicolet OMNIC software (Nicolet Ltd).

Chapter 4
Results and Discussions

4.1 Effects of nutrients

Physiochemical analysis for water samples collected from sampling sites I and II water were done at water and environmental research lab, Al-Quds university in the following years. Table 7 and table 8 present the physiochemical water analysis for Ein Sultan and Wadi Al Quilt (sampling site II and one).

Table 7: Physiochemical analysis of Ein sultan water (Water and environmental research lab, Al-Quds university)

Date	pH	Temp	NO_3^-	SO_4^{2-}	HCO_3^-	PO_4^{3-}	F^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
07/11/2001	7.25	22	17.3	19	324.1	0.21	0.25	29.6	1.82	83.2	22.4
21/10/2002	7.29	22.5	12.4	16	170.9	0.17	0.16	23.5	2.2	18.8	13.6
06/07/2003	7.39	21.4	18.3	24	305.1	*	0.16	25.3	4.1	70.5	37.6
08/12/2003	7.26	21	23	21	305.1	*	0.03	25.3	2.53	71.3	27.2
01/10/2004	7.3	21.5	21.6	23	286.8	0.19	*	22.5	2	80.2	19.4

Table 8: Physiochemical analysis of Wadi Al-Quilt water(Water and environmental research lab, Al-Quds university)

Date	pH	Temp	NO_3^-	SO_4^{2-}	HCO_3^-	PO_4^{3-}	F^-	Na^+	K^+	Ca^{2+}	Mg^{2+}
05/02/2001			15.3	34.1		N.D		17	5.57	44.7	7.5
05/03/2001	8.3		19.1	33.2		N.D		22.4	6.24	59.8	12
07/12/2001	7.37	12.5	18.4	28	207.5	0.4	0.29	25.8	3.28	34.8	7.2
02/01/2002	8.17	12.5	28.9	29	256.3	0.28	0.29	25.8	2.04	51.9	12.7
21/12/2002	6.97	11			134.2						
05/01/2003	6.85	12.1	8.6	19.6	195.3	0	0.17	29.3	7.3		

Four water samples which were collected from site I were analyzed for its (NO_3 and PO_4) seasonally during one year (2006-2007).

Date of samples collection, sampling sites, their nutrient contents are shown in table 9.

Table 9: Nutrient levels of water samples collected from sampling site I.

Sample No.	Sample Date	Sampling site	NO_3 Concentration (mg/L)	PO_4 Concentration (mg/L)	Volume of filtered water (mL)	Microscopic results	FTIR result
1	22/1/2006	Site I			1000	Not Detectable	Oscillatoria fingerprint
2	24/2/2006	Site I	3.4	0.1	1000	Not Detectable	Oscillatoria fingerprint
3	19/3/2006	Site I			1000	Oscillatoria	Oscillatoria fingerprint
4	24/4/2006	Site I	3.9	0.3	500	Oscillatoria	Oscillatoria fingerprint
5	22/5/2006	Site I			500	Oscillatoria	Oscillatoria fingerprint
6	26/6/2006	Site I			300	Oscillatoria	Oscillatoria fingerprint
7	10/7/2006	Site I	5.8	1.29	100	Oscillatoria	Oscillatoria fingerprint
8	24/8/2006	Site I			100	Oscillatoria	Oscillatoria fingerprint
9	23/9/2006	Site I			100	Oscillatoria	Oscillatoria fingerprint
10	3/10/2006	Site I	4.1	0.3	300	Oscillatoria	Oscillatoria fingerprint
11	27/11/2006	Site I			500	Oscillatoria	Oscillatoria fingerprint
12	20/12/2006	Site I			1000	Oscillatoria	Oscillatoria fingerprint
13	23/1/2007	Site I			1000	Oscillatoria	Oscillatoria fingerprint
14	8/3/2007	Site II			500	Oscillatoria	Oscillatoria fingerprint
15	14/3/2007	Site III			500	Different kinds of Invertebrates	Different fingerprint.

Results showed one kind of cyanobacteria have been identified as a predominant species in site I and site II. This species have been identified as Oscillatoria. It was found that the abundance of Oscillatoria was directly proportional with nutrient concentration. This result complies well with the result of Douterelo (Douterelo, et al., 2004).

The correlation between Oscillatoria abundance and the nutrient content reflected that this type of cyanobacteria were predominant because of the high concentration of nutrient

Nutrient levels in this aquaculture pond were higher during summer, fall than during spring and winter (Figure 7, Figure 8). Different volumes of water sample were filtered in order to get the same amount of Oscillatoria powder (0.4 mg of solid sample), large volume in winter (about 1 litter) while very small volume during summer (about 100 ml). This variation in Oscillatoria abundance is mainly due to two main reasons, the first reason is the variation in PO_4 and NO_3 concentration, the second main reason is temperature. High Temperature during summer has a direct effect on Oscillatoria abundance and vice versa. Low concentration of Oscillatoria in rainy seasons due to low temperature. Decrease sun light and low temperature inhibited the reproduction and growth of Oscillatoria while in dry seasons large amount of sun light, high temperature will cause large amount of photosynthesis so large amount of Oscillatoria in water (Douterelo, et al.,2003).

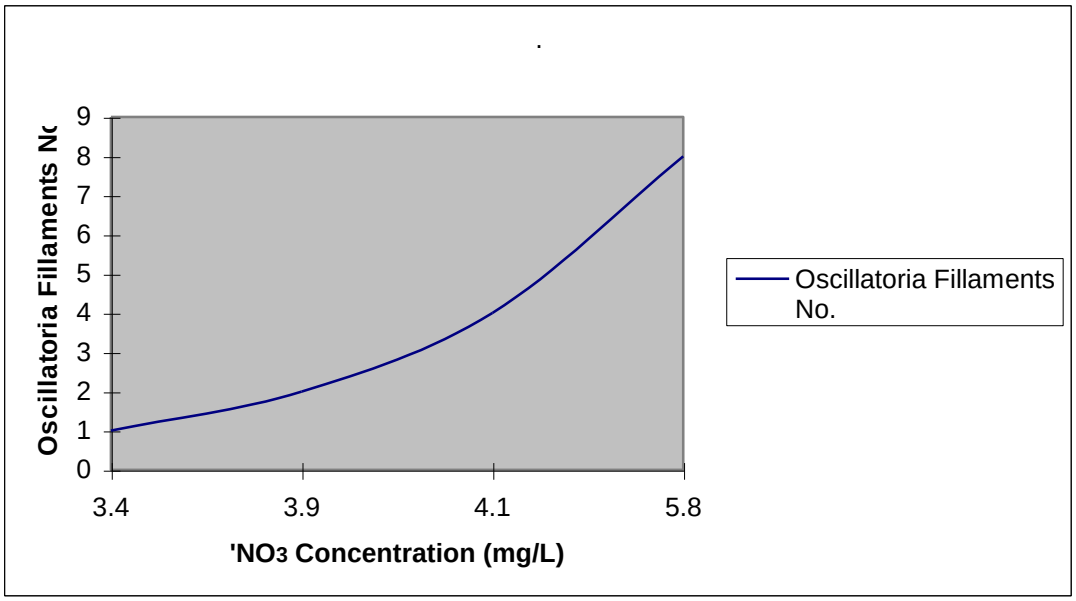

Figure 7: Correlation between NO_3 concentration in water samples collected from site I and Oscillatoria filament.

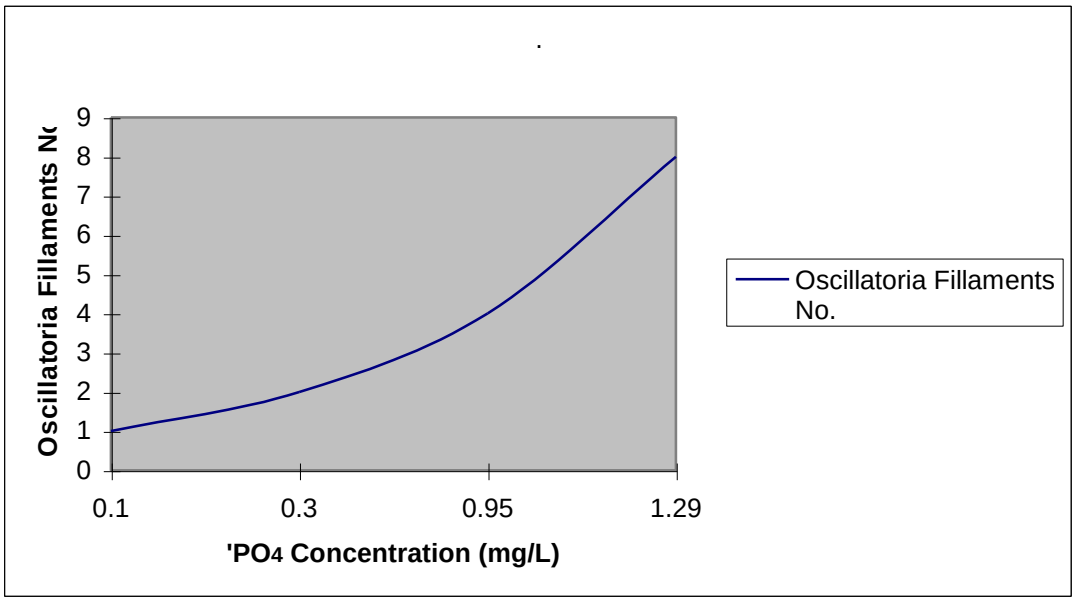

Figure 8: Correlation between PO_4 concentration in water samples collected from site I and Oscillatoria filament.

The Jericho Aquaculture pond (site I) is characterized by a continues inflow of water from wadi Alquilt. Water is also moving outflow in a continuous motion. Inside the pond the water is clear. All preparations of micro samples showed only Oscillatoria blue green algae. The short resident time for the water and the judicious mixing is the main reason for the non diverse algae community. Oscillatoria is domination in clear nutrient fertilized water (Douterelo, et al., 2003). Mixing due to continuous motion will result in short periods of stabilization that may prevent build-up of very large crops of any algae by changing the environment frequently enough to prevent any particular group reaching its maximum potential. Blue green algae blooms are now frequent and even a small bloom can be concentrated by wind at the edge of a pond to form a hazard.

In drinking water reservoirs fed by fertile water, large growth of algae cause problem because filtration to remove the algae , particularly blue-green algae, and produce clear sparkling water is expensive, plus a realization that many blue-green algal populations are toxic, has led to considerable interest in their ecology. Blue green algae not only may be toxic but may produce noxious 'pigsty' tastes if they decompose on filters used for clearing the water.

4.2 Qualitative test

4.2.1 Microscopic screening

Wet mount preparations of water samples showed the same type of cyanobacteria which was Oscillatoria present in sampling site I, sampling site II (Figure 9) while different kinds of invertebrates (Daphnia, euglena, paramecium, etc) were found in sampling site III .

Variable concentrations of Oscillatoria were found during the year 2006-2007 in site I. Very low concentration in rainy seasons due to decrease in sunlight and temperature which are needed for Oscillatoria abundance and growth while it was had high concentrations in summer and spring. (Hoak, et al., 1995). Oscillatoria was very difficult to be identified during rainy seasons because it was with very low concentration.

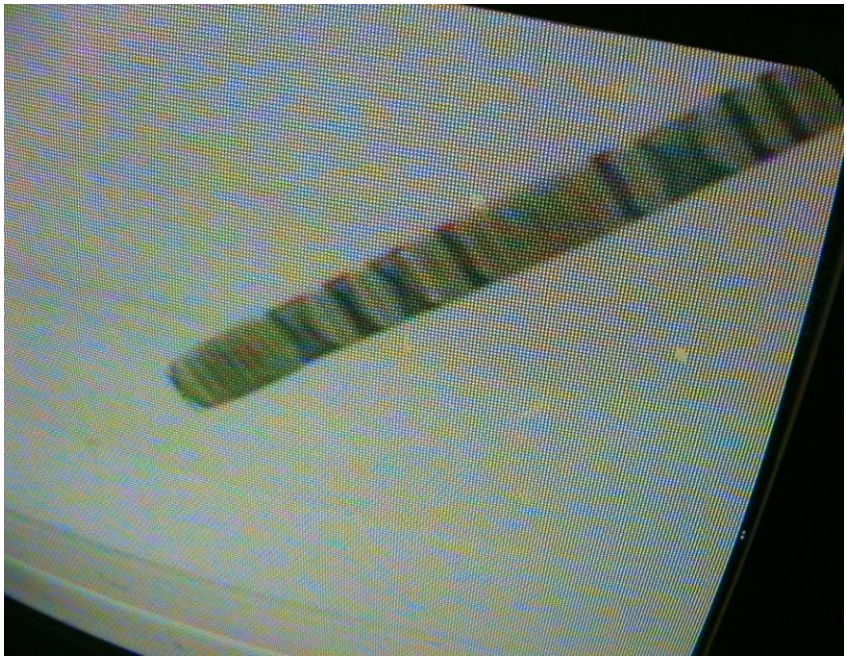

Figure 9: Oscillatoria under microscope.

4.2.2 Oscillatoria standard

FTIR spectra of Oscillatoria showed a well defined sequence of bands, band attribution is based on the preparation of Oscillatoria standard (Figure 10). A standard of Oscillatoria was prepared by isolating one filament under microscope .The filament was returned to the same filtered water sample, which contains no microorganisms. Growth was enhanced using Sunlight. The closed vial was incubated at room temperature for about one week. Filter paper with small pores (small pores 0.45 µm) was used in order to eliminate all microorganisms that were found in the water sample because their size is larger than the filter paper pores (Keeton, 1983). The water sample was screened under the microscope before and after Oscillatoria filament was incubated when water sample in the closed bottle was screened again using the microscope only Oscillatoria was presented, the sample was filtered and prepared for FTIR analysis.

Oscillatoria standard spectrum was used as a reference for all of the water samples which were collected from the three different sampling sites. When all FTIR results of all water samples which were collected from the three sampling sites were compared with Oscillatoria standard

fingerprint results showed that all water samples which were collected from sampling site I and II had very similar spectrum (Figure 15), While water sample which was collected from sampling site III had totally different spectra (Figure 17).

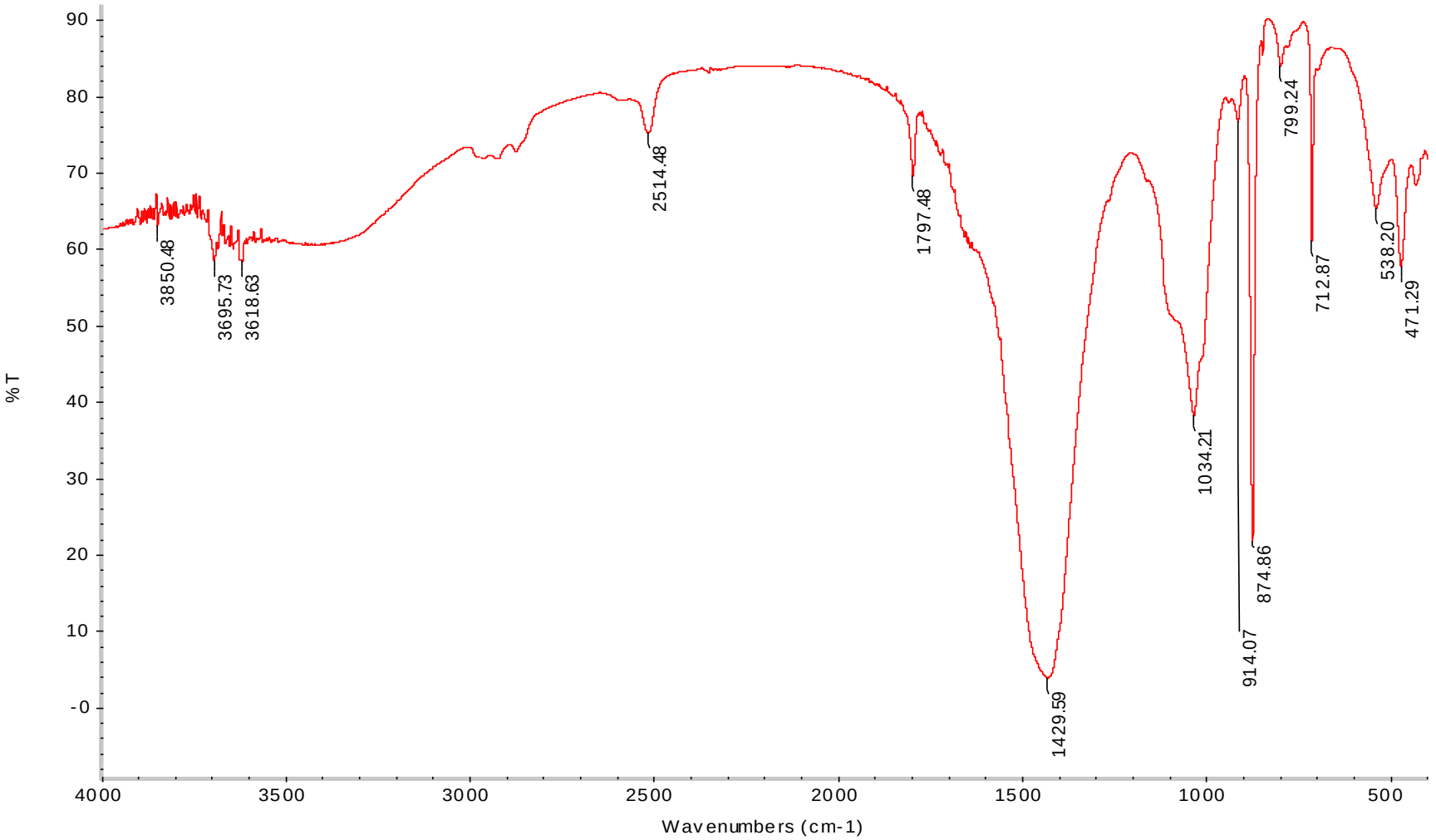

Figure 10: The infrared spectra of Oscillatoria Standard in the 4000-500 cm^{-1} region .

FTIR band assignments of Oscillatoria follow the general trends in literature (Dudley, et al., 1987, Scheinmann, 1970) with characteristics in molecular vibration to different wave number intervals (Table 8).

Table 10: General band assignment of Oscillatoria (Dudley etal., 1987, Scheinmann, 1970)

Peak Number	Peak wave Number(cm^{-1})	Tentative assignment of bands	Description
1	3850	O-H, N-H stretching	Very weak
2	3695	C-H stretching	Very weak
3	3619	O-H stretching	Very weak
4	2614	NH_2^+ asymmetric ,and symmetric stretching	weak
5	1797	C=C stretching	Weak, sharp
6	1429	CH_2 bending	Very strong, diffused
7	1034	C-O stretching	Strong, sharp
8	914	$=CH_2$ out of plane bending	Very weak
9	874	N-H out of plane bending	Very strong, very sharp
10	799	C-H out of plane bending	Very weak
11	712	Unidentified peak	Strong, very sharp
12	538	C-Br and C-I stretching	Very weak, sharp
13	471	Of no practical value	Weak, sharp

4.2.3 Negative control preparation

As a negative control , water sample number 9 which was collected at 23/9/2006 from sampling site I and contains Oscillatoria when it was screened by the compound microscope was autoclaved for about 15 minutes in order to kill Oscillatoria (all chemical functional groups were destroyed) (Sigee, et al.,2001), FTIR analysis results showed no spectrum (Figure 11). A negative control was needed in our work to be sure that the fingerprint which was obtained when the water sample was analyzed using FTIR was for Oscillatoria and not for any other pollutants.

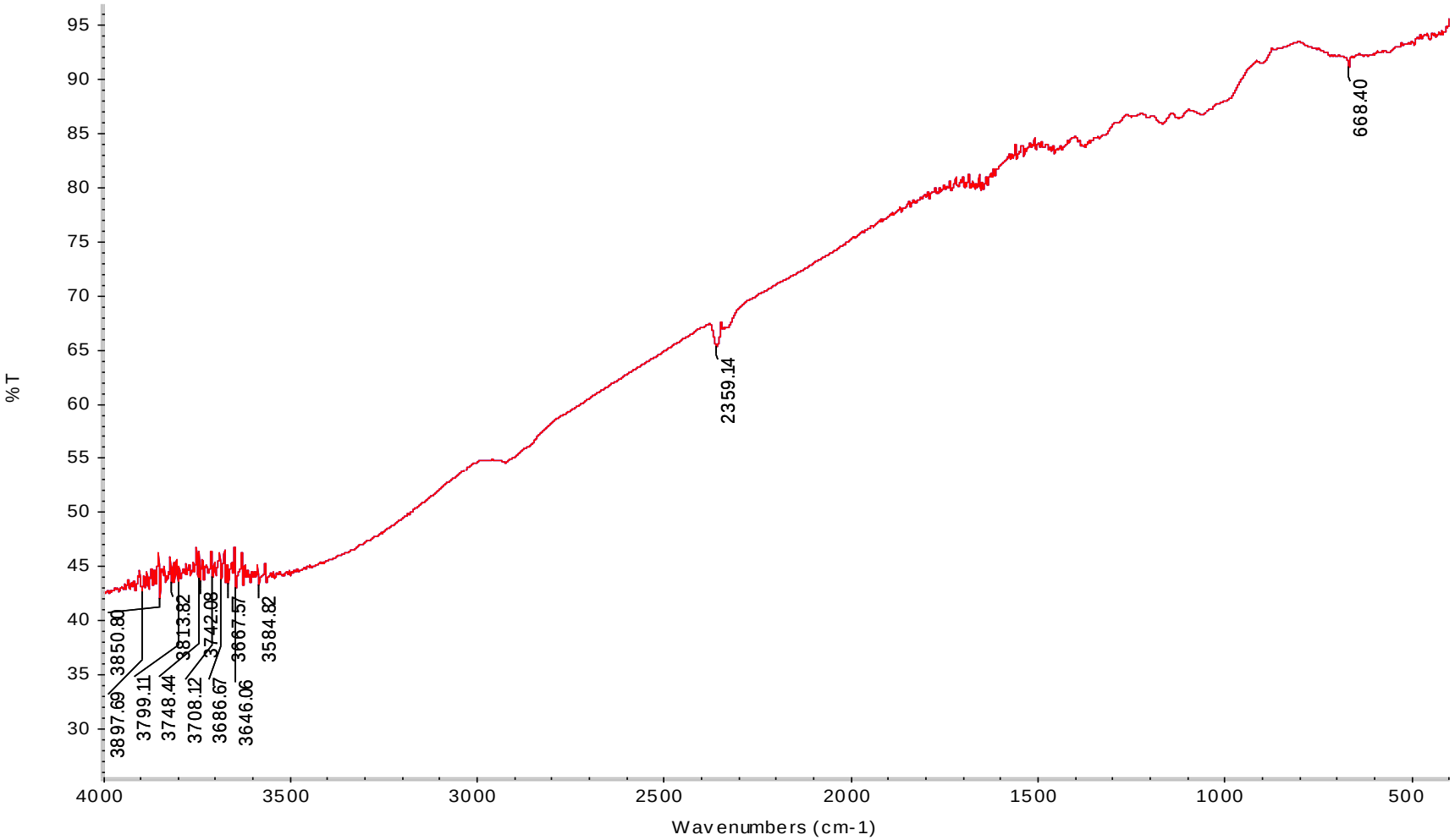

Figure 11: The infrared spectra of Autoclaved Oscillatoria in the 4000-500cm^{-1}region.

4.2.4 FTIR results

Oscillatoria cell membrane contains a different set of proteins that have different chemical composition which will specialize it from other type of microorganisms (Albert et al., 2004). Oscillatoria fingerprint show these chemical functional groups series (Dudley, et al., 1987). These sets are easily detected by FTIR (Jose, et al., 1999).

Low density or low abundance Oscillatoria population is difficult to identify under the microscope. This was the case in the aquacultures pond (sample site I) during fall and winter. On the other hand these microorganisms were directly identified using their fingerprint FTIR spectra. Large and large volume of filtered water will result in the appearance of this signal that is characteristic for this microorganism. This means that a safe level could be assigned for a special volume of water. This may add to the validity and reproducibility of FTIR water technology (Sandt, et al., 2002).

4.3 The Effect of overlapping on Oscillatoria fingerprint

4. 3.1 Escherichia coli (E.coli)

E.coli was analyzed by FTIR in order to be compared by Oscillatoria fingerprint. Results showed different fingerprint for E.coli with no overlapping with Oscillatoria fingerprint (Figure 12 ,Figure 13).

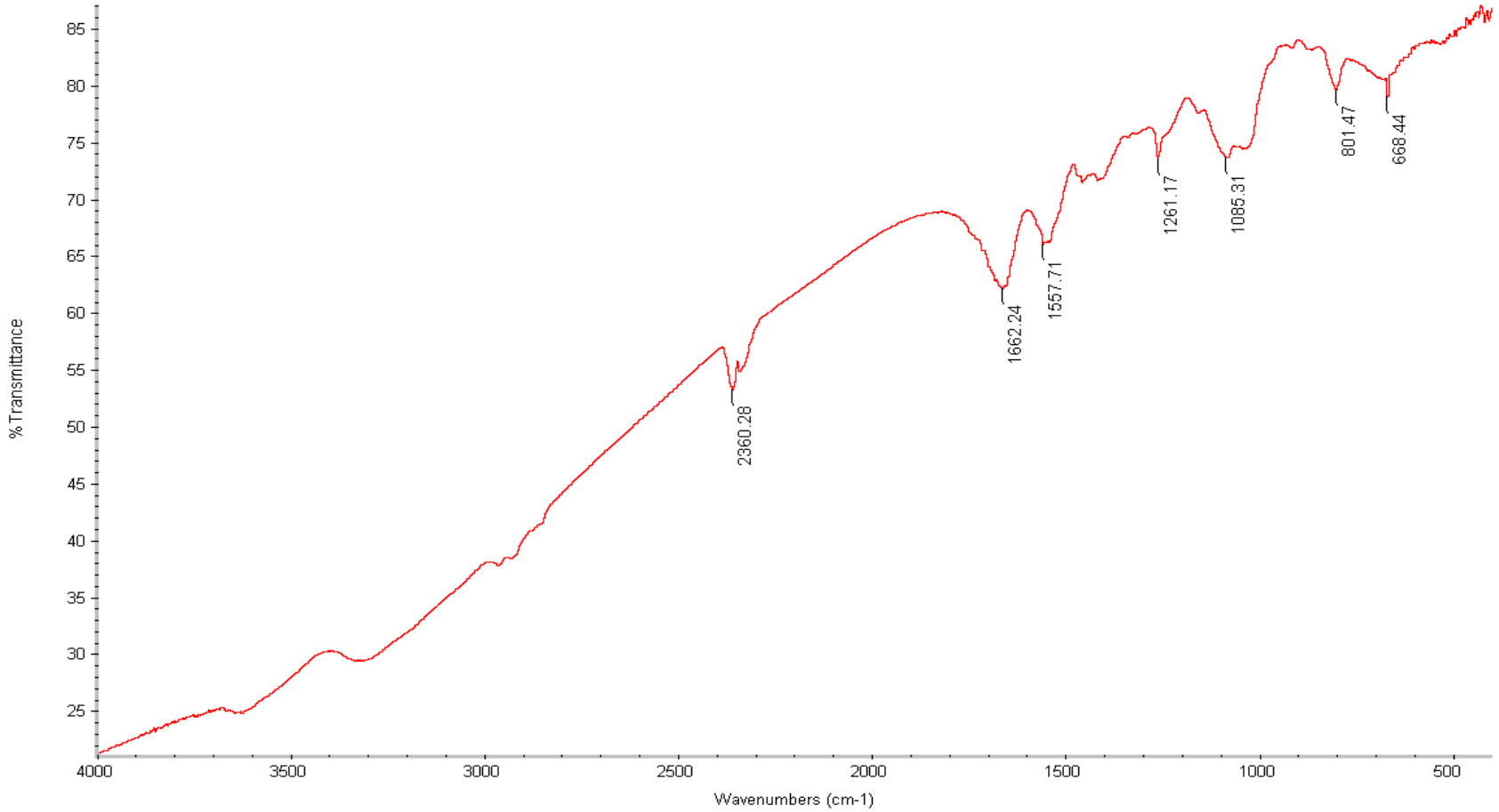

Figure 12: The infrared spectra of E.coli in the 4000-500 cm^{-1} region.

Table 11: General band assignment of E.coli (Dudley, et al., 1987, Scheinmann, 1970)

Peak No	Peak wave number(cm^{-1})	Tentative assignment of bands	Description
1	2360	O=C=O	Very weak, sharp
2	1662	C=C	Very weak, diffused
3	1557	C-O antisymmetrical and symmetrical stretching respectively	Very weak, diffused
4	1261	O-H bending	Very weak, sharp
5	1085	C-O stretching	Very, weak diffuse
6	801	N-H out of plane bending	Very weak, diffused
7	668	C-H out of plane deformation	Very weak, sharp

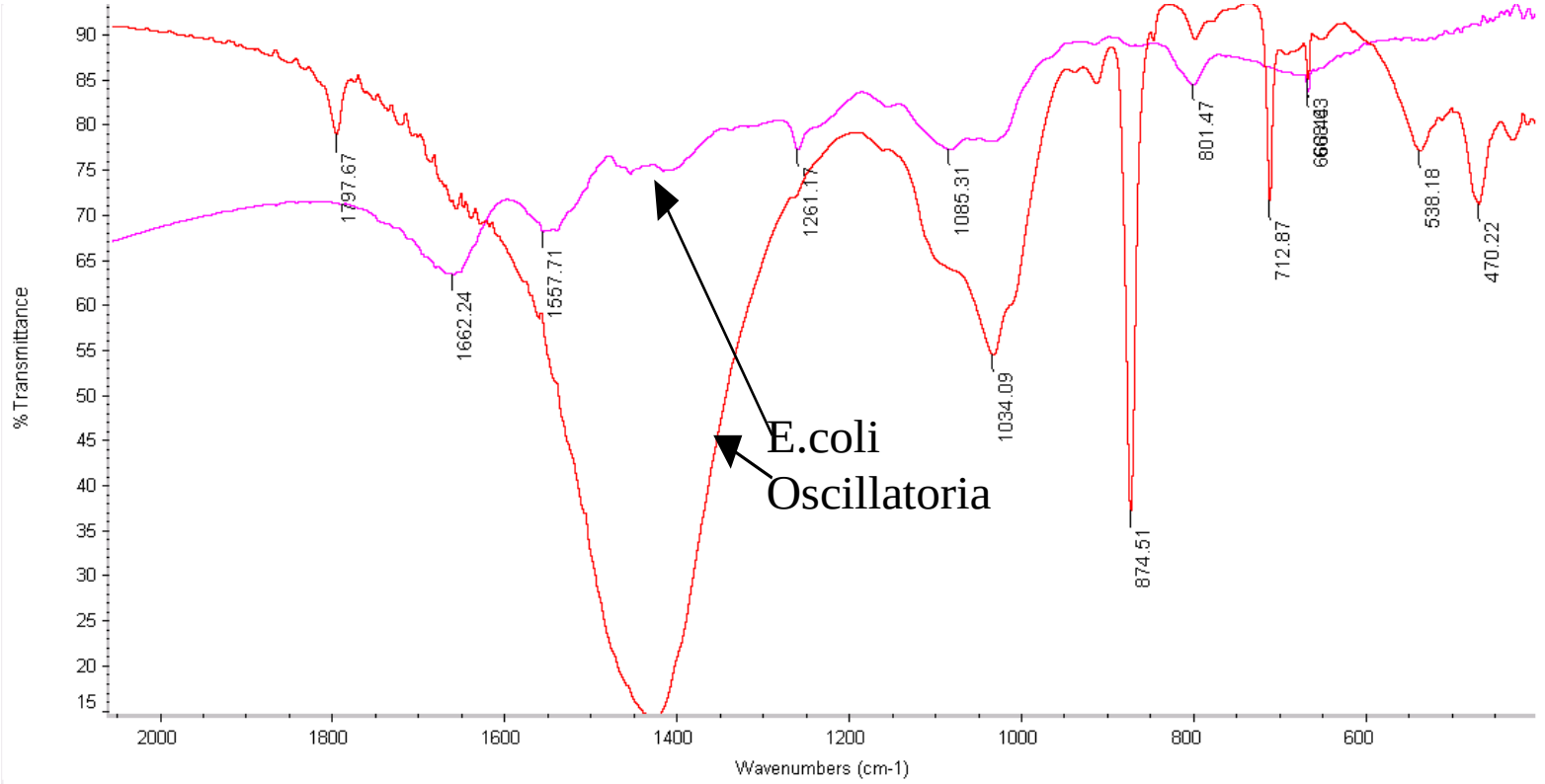

Figure 13: The infrared spectra of Oscillatoria and E.coli in the 2000-500 cm –1 region.

4.3.2 The effect of filter paper porosity

In order to study the probability of overlapping of the Oscillatoria fingerprint with other types of microorganisms that are found in the same water sample, water sample No.14 which was collected from site II at 8/3/2007 was filtered using two different whatman filter paper, the first one with 2.7µm pores size to eliminate only Oscillatoria filaments because they have larger size than the filter paper pores size which is about 50 µm (Graham, et al., 2000). When it was compared with Oscillatoria standard FTIR results showed the same fingerprint (Figure 14, Figure 15).

The second filter paper with 0.45µm pores size was used to filter the same water sample .All microorganisms founded in the water sample including Oscillatoria, bacteria and small invertebrate were eliminated on the filter paper because all of them are larger in size than the filter paper pores size (Keeton, 1983). When it was compared with Oscillatoria standard FTIR results showed a fingerprint which contained additional peaks reflecting functional groups of the other types of microorganisms founded in the water sample (Figure 14, Figure 15).

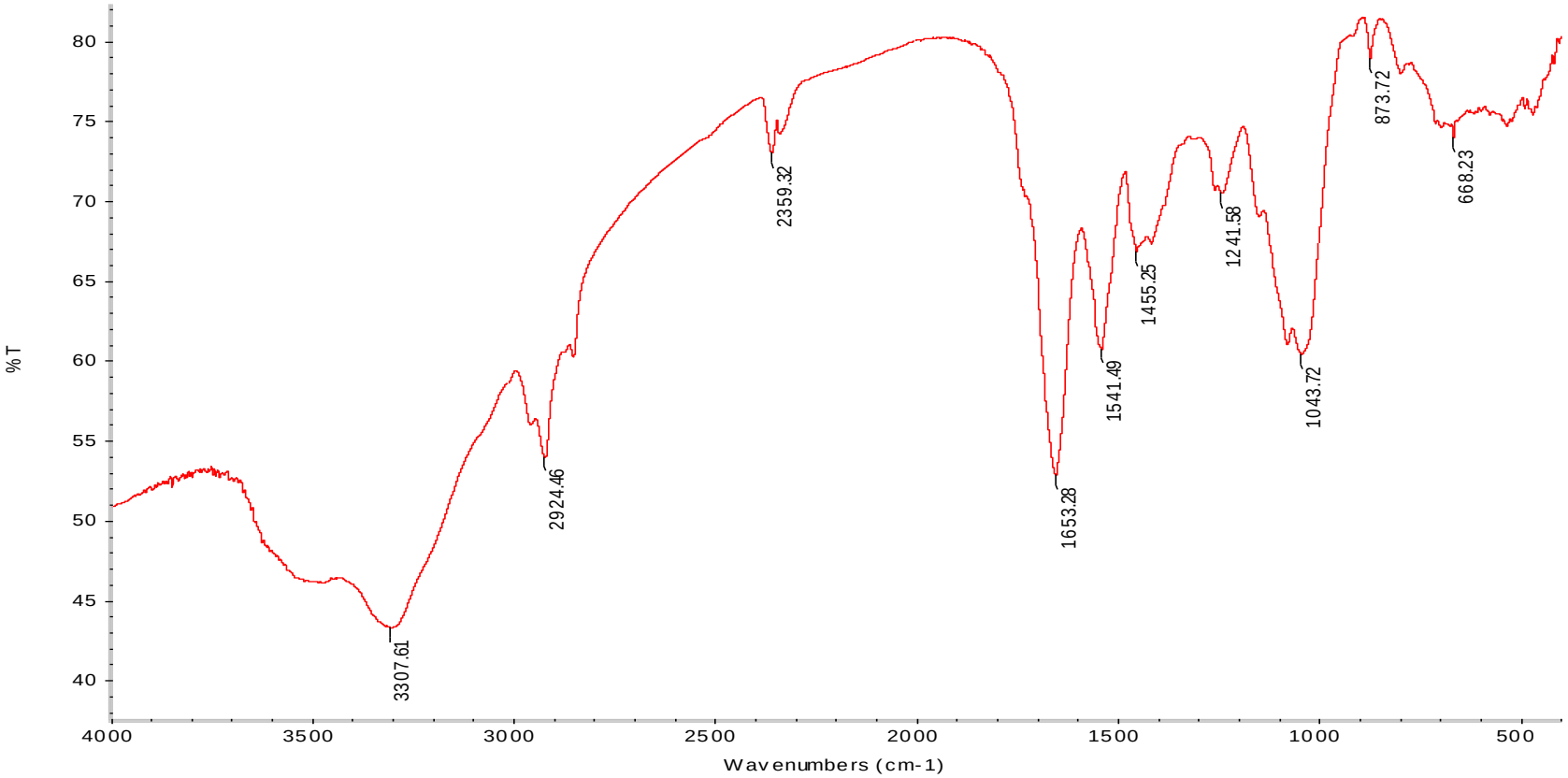

Figure 14:The infrared spectra of Oscillatoria which was collected from site II using 0.45 um filter paper pores in the 4000-500 cm-1 region.

Table 12: General band assignment of sampling II water sample using 0.45um filter paper (Dudley, et al., 1987, Scheinmann, 1970)

Peak No	Peak wave number(cm^{-1})	Tentative assignment of bands	Description
1	3307	N-H stretching lowered on H bonding	Strong, diffused
2	2924		Weak, sharp
3	2359	O=C=O	Very weak. sharp
4	1653	Amide I -NH_3-	Very strong, very sharp
5	1541	Amide II found in open chain	Strong, very sharp
6	1455	-N=N- asymmetric and symmetric stretching	Diffused, weak
7	1241	C-O stretching	Diffused, weak
8	1043	C-O stretching	Diffused, strong
9	873	N-H out of plane bending	Very weak, very sharp
10	668	C-H out of plane deformation	Very weak

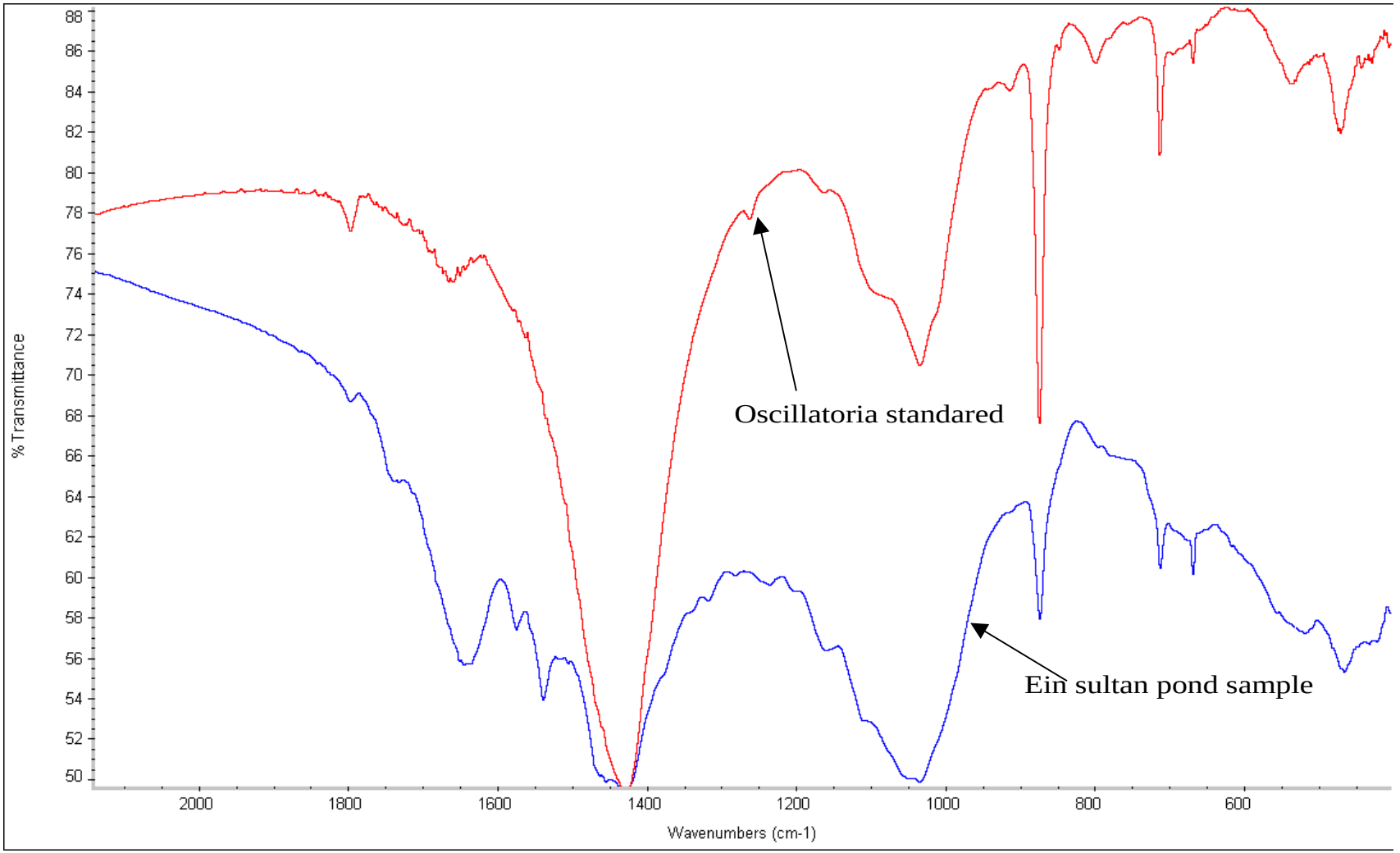

Figure 15:The infrared spectra of Oscillatoria standard and Oscillatoria collected from site II in the 2000-500 cm –1 region

4.4 The distribution of Oscillatoria in West Bank

In order to study the distribution of Oscillatoria in the West Bank, two water samples, sample No.14 (which was collected from sampling site II at 8/3/2007) and sample No.15 (which was collected from sampling site III at 14/3/2007) were analyzed using FTIR. Comparing the fingerprint of each water sample with Oscillatoria standard fingerprint. It was found that

The fingerprint of sample 14 is the same as Oscillatoria standard fingerprint (Figure 19, Figure 20). While different fingerprint was obtained for sample 15 (Figure 17, Figure 18). The main reasons for the dominance of Osillatoria in water samples collected from Jericho region are climate conditions which are suitable for Oscillatoria which has optimal temperatures for growth ranges from $15C^{o}$-$35C^{o}$ (Hoak, et al., 1995) rather than Bethlehem region.

The climate of Jericho is arid, which is hot in summer and warm in winter, January is the coldest month with an average of $19C^{o}$ and August is the hottest month with an average of $38C^{o}$ while Bethlehem climate which is cold in winter, with high of 13 to low of 1 C^{o} in January and, moderate in summer with high of 27 to low of 17 C^{o} in August.

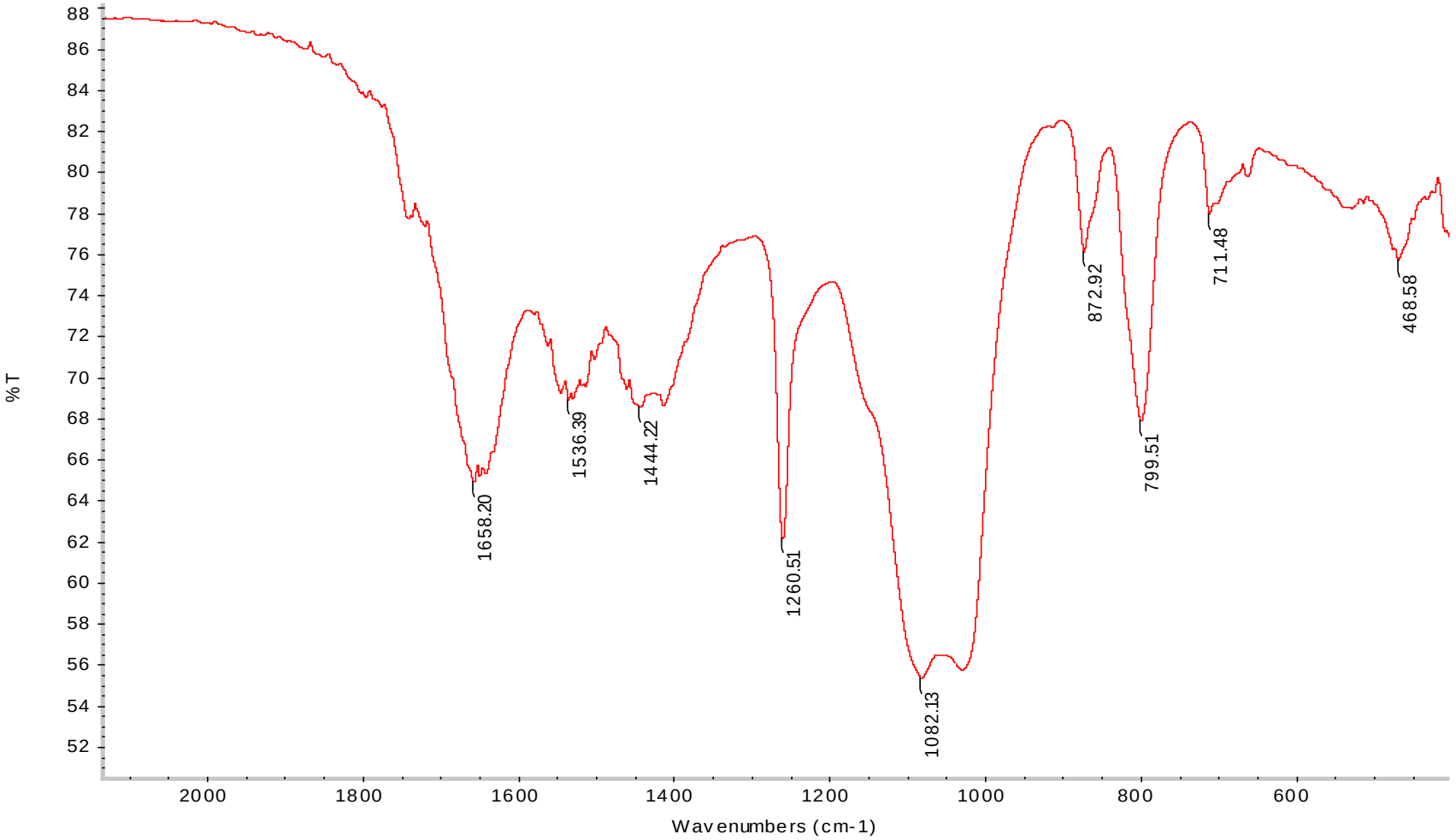

Figure 16: The infrared spectra of microorganisms which was collected from site III in the 2000 -500 cm-1 region.

Table 13: General band assignment of sampling III water sample (Dudley, et al., 1987, Scheinmann, 1970)

Peak No	Peak wave number(cm-1)	Tentative assignment of band	Description
1	1658	$-NH_3$	Diffused, strong
2	1536	Amide II found in open chain only-CONH-	Diffused weak
3	1444	-N=N- asymmetric and symmetric stretching	Diffused weak
4	1260	O-H bending	Very sharp very strong
5	1082	C-O stretching	Diffused, very strong
6	872	N-H out of plane bending	Strong sharp
7	799	C-H out of plane bending	Very strong very sharp
8	711	Unidentified peak	Sharp very weak
9	468	Of no practical value	Sharp very weak

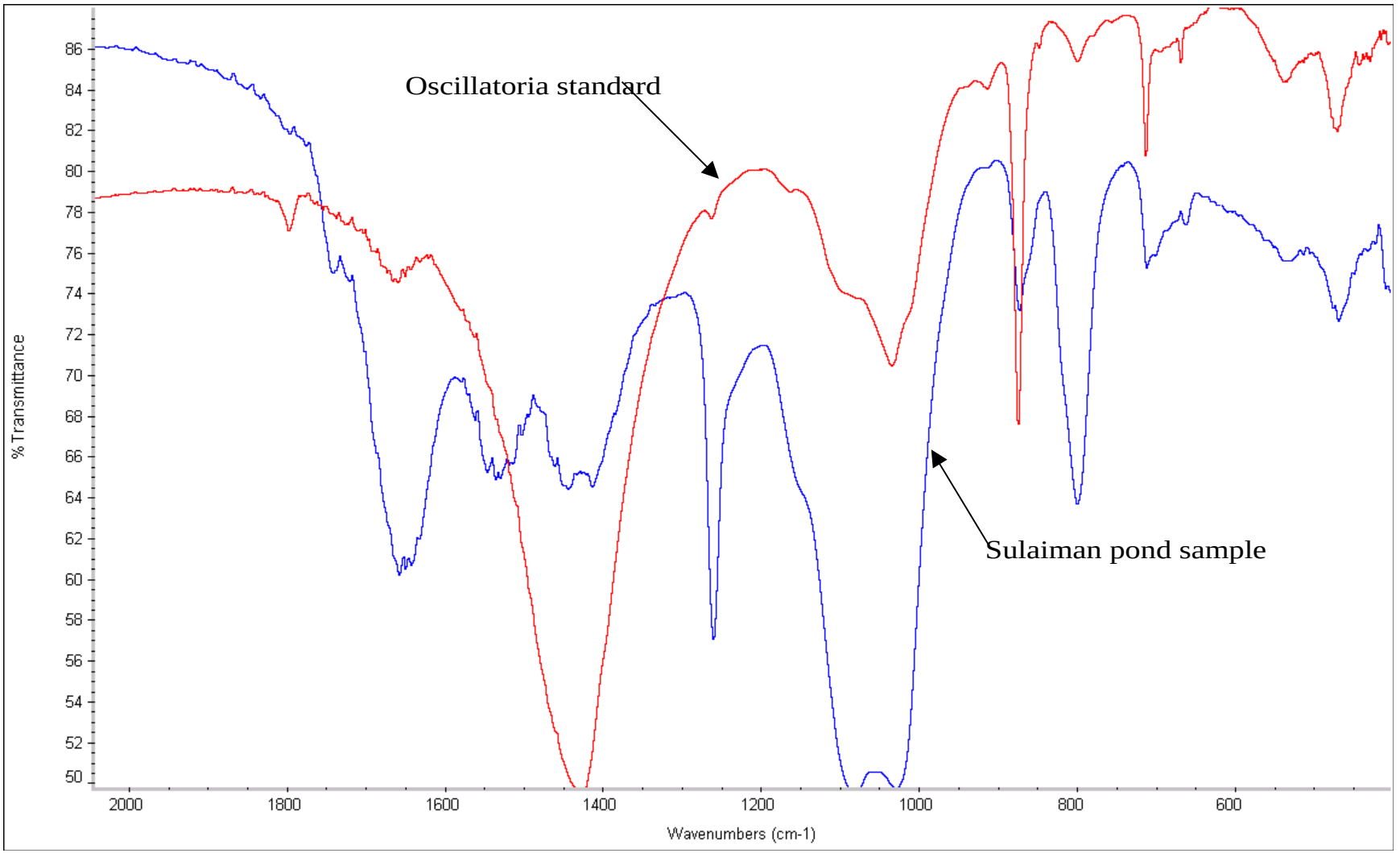

Figure 17: The infrared spectra of Oscillatoria standard and microorganisms collected from site III in the 2000-500 cm –1 region

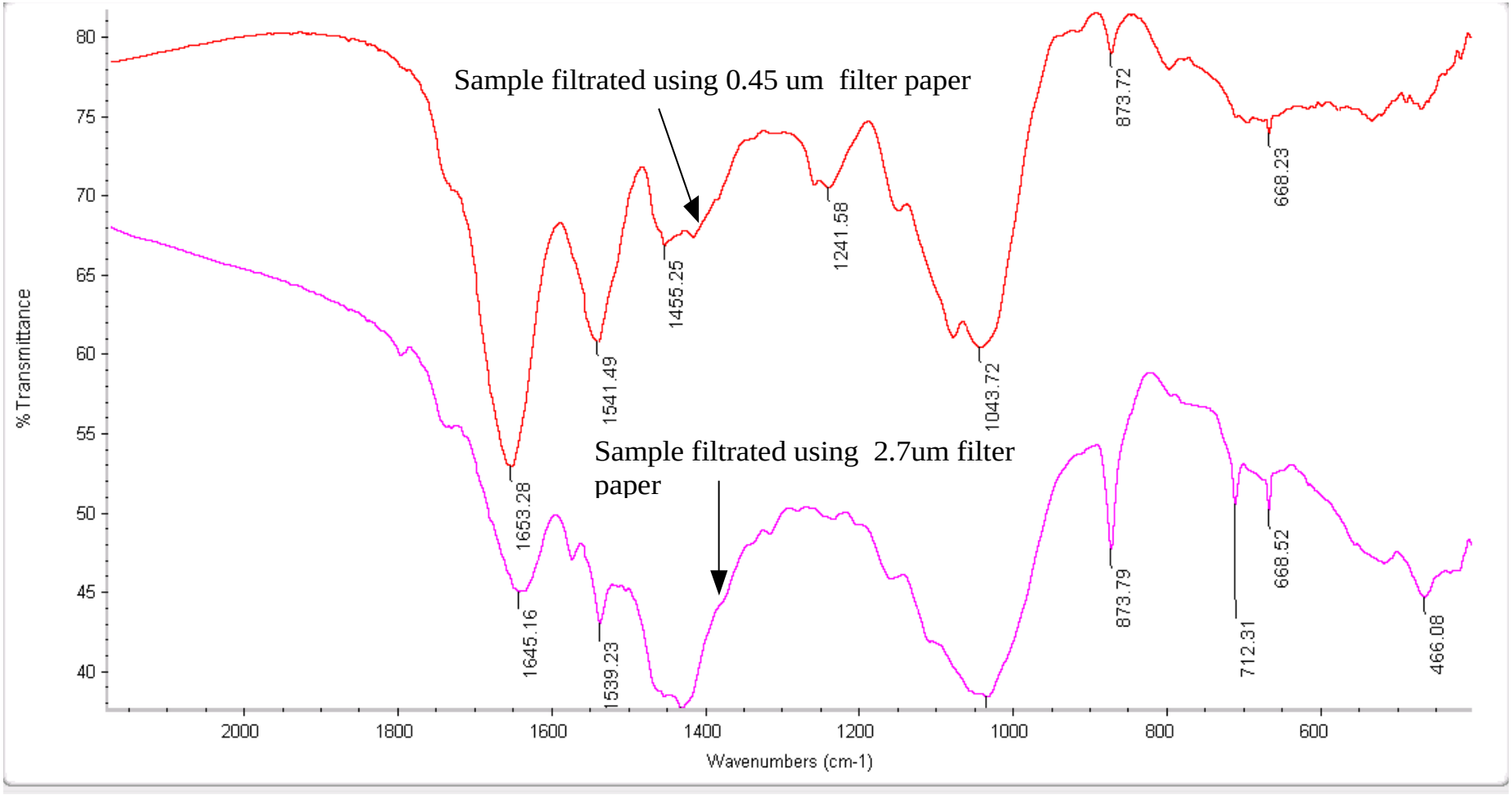

Figure 18: The infrared spectra of the same water sample which was collected from site II using two different filter paper pores.

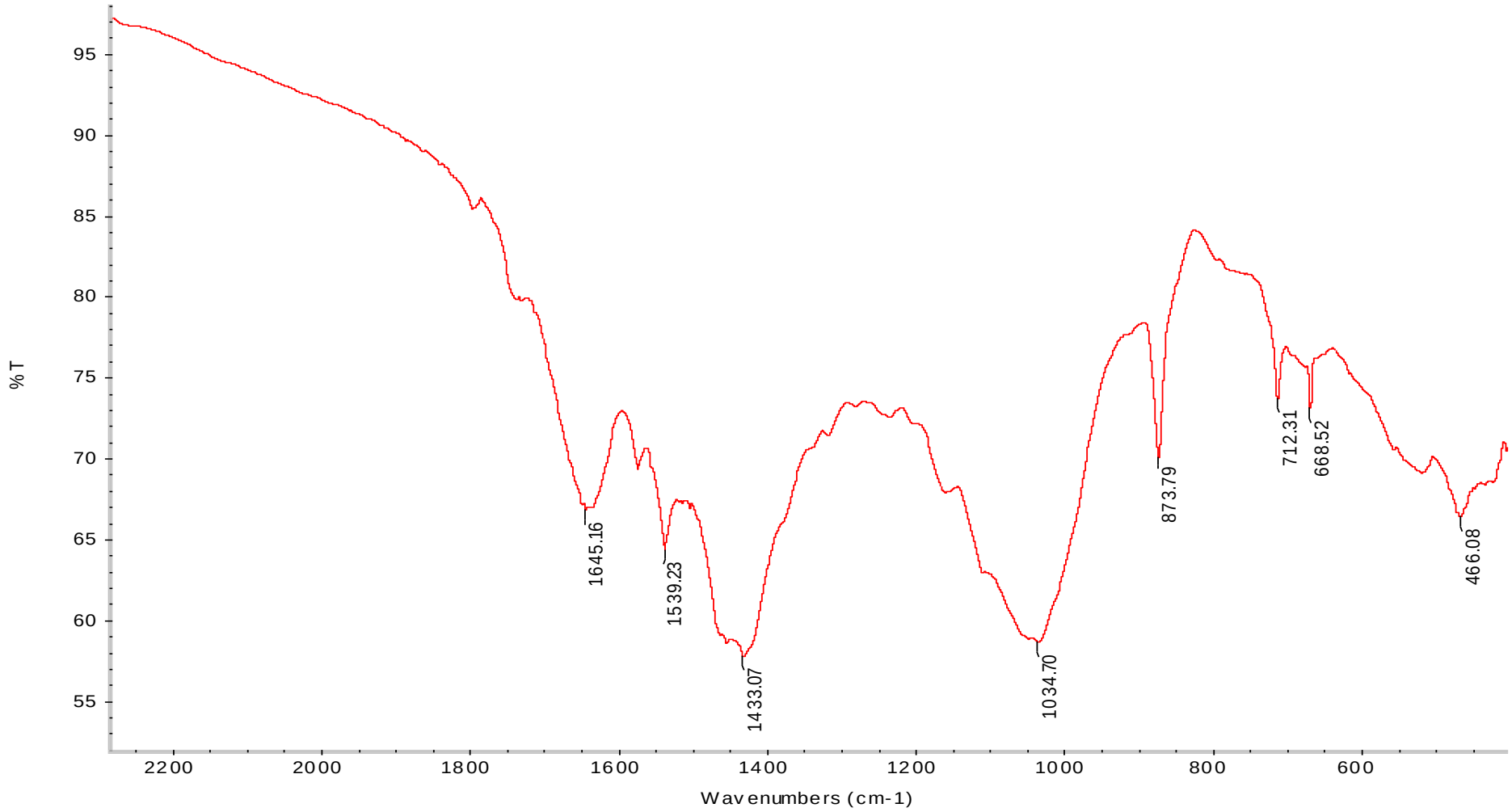

Figure 19:The infrared spectra of Oscillatoria which was collected from site II using 2.7µm filter paper in the 2200 -500 cm-1 region.

Table 14: General band assignment of sampling II water sample using 2.7 um filter paper (Dudley, et al., 1987, Scheinmann, 1970)

Peak No	Peak wave number(cm-1)	Tentative assignment of band	Description
1	1645	$-NH_3$	Diffused weak
2	1539	AmidII -CONH- in open chain amid only	Sharp weak
3	1433	$=CH_2$	Diffused strong
4	1034	C-O stretching	Diffused very strong
5	873	N-H out of plane bending	Very sharp, very strong
6	712	Unidentified peak	Sharp weak
7	688	C-H out of plane deformation	Sharp weak
8	466	Of no practical value	Diffused very weak

4.3 Quantitative tests

Water sample No.8 which was collected from sampling site I at 30/8/2006. This water sample was firstly screened by the compound microscope. Results of microscopic screening showed Oscillatoria filaments. The water sample was then exposed to sunlight in order to enhance Oscillatoria growth and proliferation for about one week from 30/8/2006-6/9/2006. About 100ml from this sample were filtered daily using Whatman filter paper with 2.7um pores to isolate only Oscillatoria filaments which have larger size than the pores of the filter paper .All these water samples were also analyzed daily by FTIR for one week. Table 15 shows the quantitative test results:

Table 15: Quantitative test results(+ : Oscillatoria filament number increased,- :Transmittance decreased)

Sample No.	No. of exposure days	Volume of filtered water (mL)	Microscopic results(Oscillatoria filament number)	FTIR result (Transmittance %)
8	1 (30/8/2006)	100	+	-
	2 (31/8/2006)	100	++	--
	3 (1/9/2006)	100	+ + + +	- - - -
	4 (2/9/2006)	100	+ + + + + +	- - - - - -
	5 (3/9/2006)	100	+ + + + + + + + +	- - - - - - - -
	6 (4/9/2006)	100	+ + + + + + + + + + + + +	- - - - - - - - - - -
	7 (5/9/2006)	100	+ + + + + + + + + + + + + + + + +	- - - - - - - - - - - - - -- -

Oscillatoria abundance was increased when it has been incubated under sunlight for about one week as has been directly indicated by the microscope. During this incubation period an FTIR spectrum was obtained for this culture on a daily bases (Figures 20, 21, 22). FTIR spectrum of these daily samples showed an increase in FTIR signals during the incubation period (the transmittance was decreased because the Absorbance was higher due to the high concentration) this mean that the quantity or abundance of Oscillatoria sample was increased

This result showed that FTIR is excellent method not only for qualitative determination of microorganisms found in water but also for quantitative analysis of microorganisms which means that it can be used for exact determination of the water quality and its uses (Sigee et al., 2001).

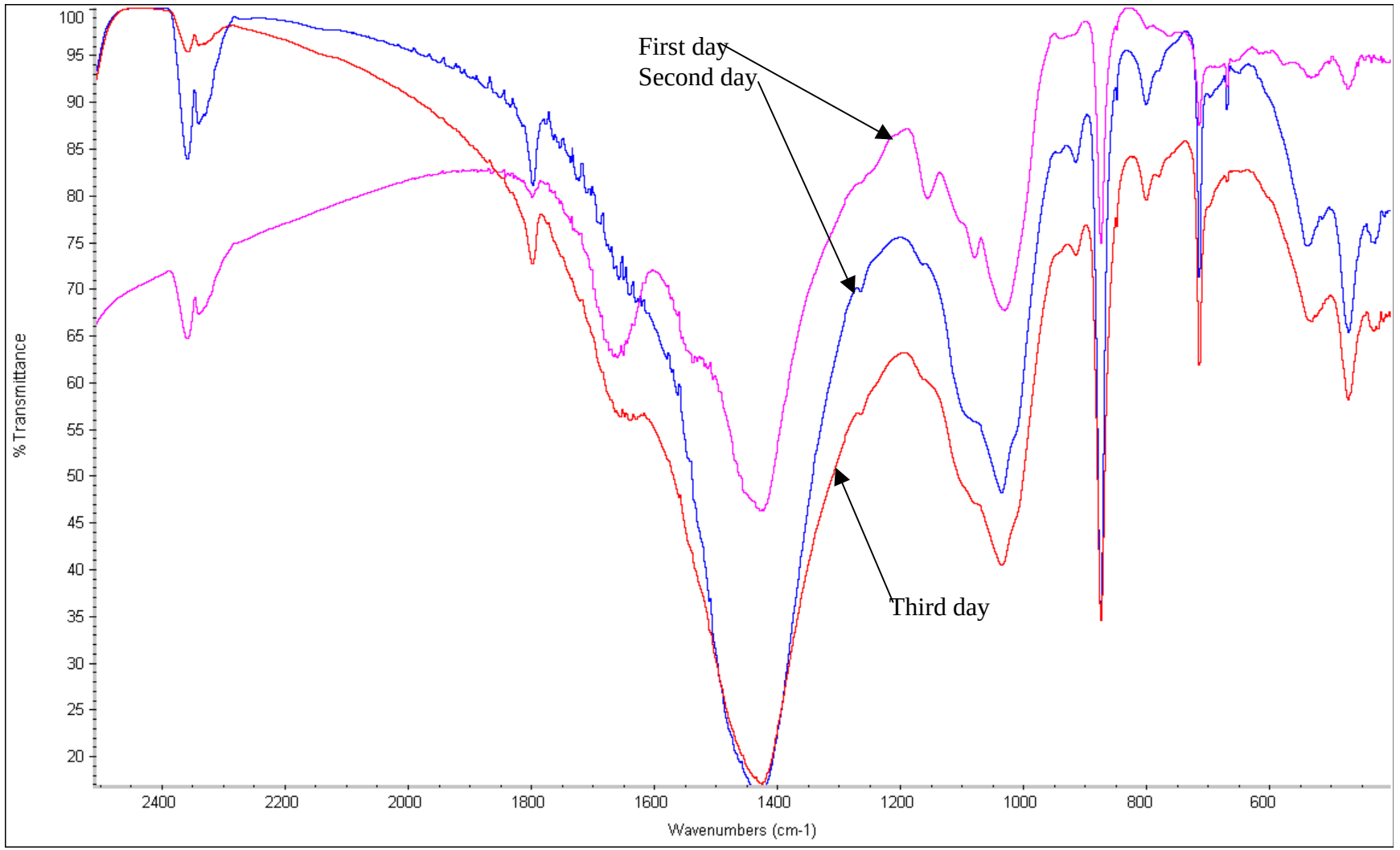

Figure 20:The infrared spectra of Oscillatoria showing correlation between the increase of its amounts by sun light exposure period and transmittance decreasing in the 2400-500 cm-1 region(First, Second and Third).

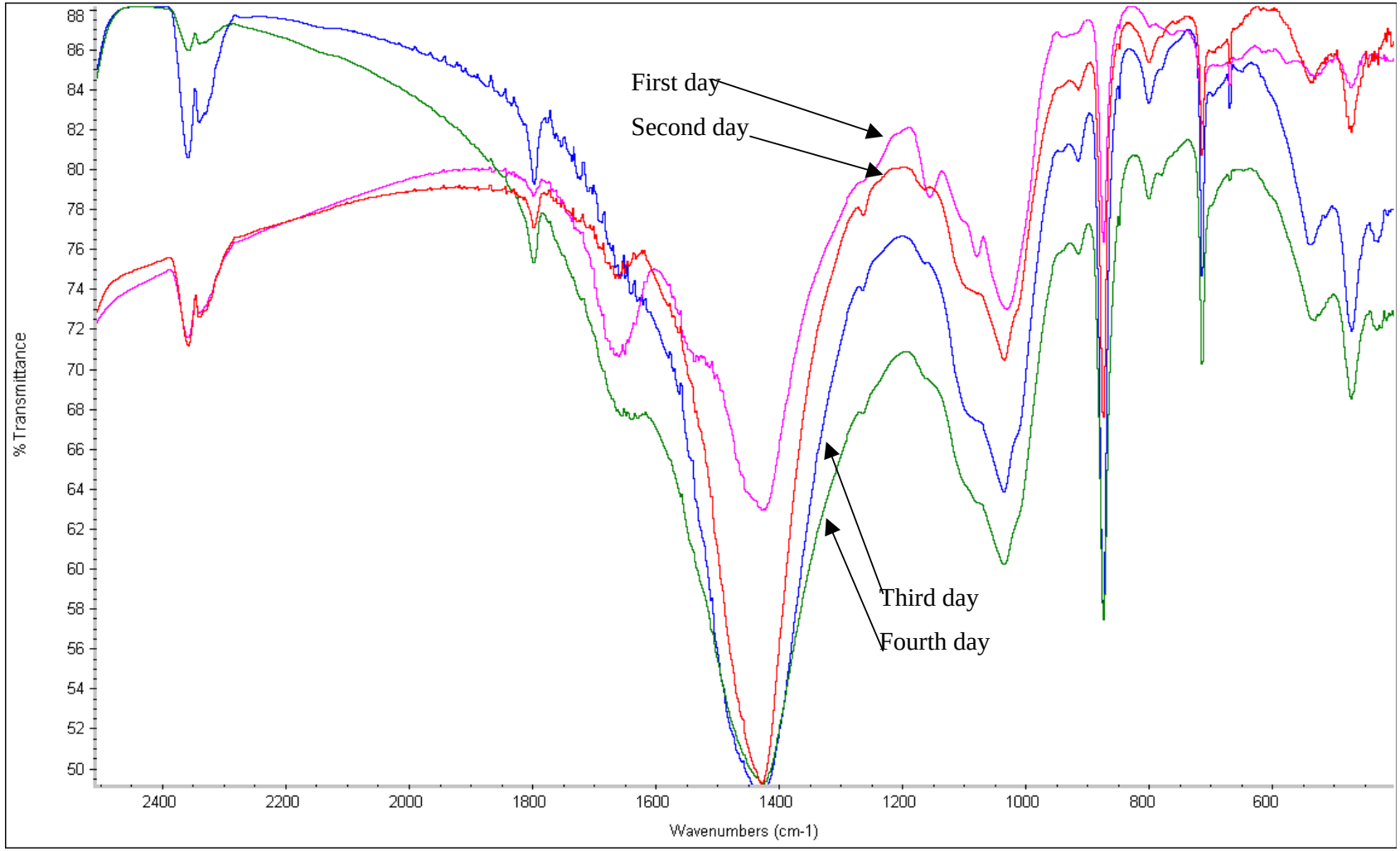

Figure 21:The infrared spectra of Oscillatoria showing correlation between the increase of its amounts by sun light exposure period and transmittance decreasing in the 2400-500 cm-1 region(First, Second ,Third and Fourth day).

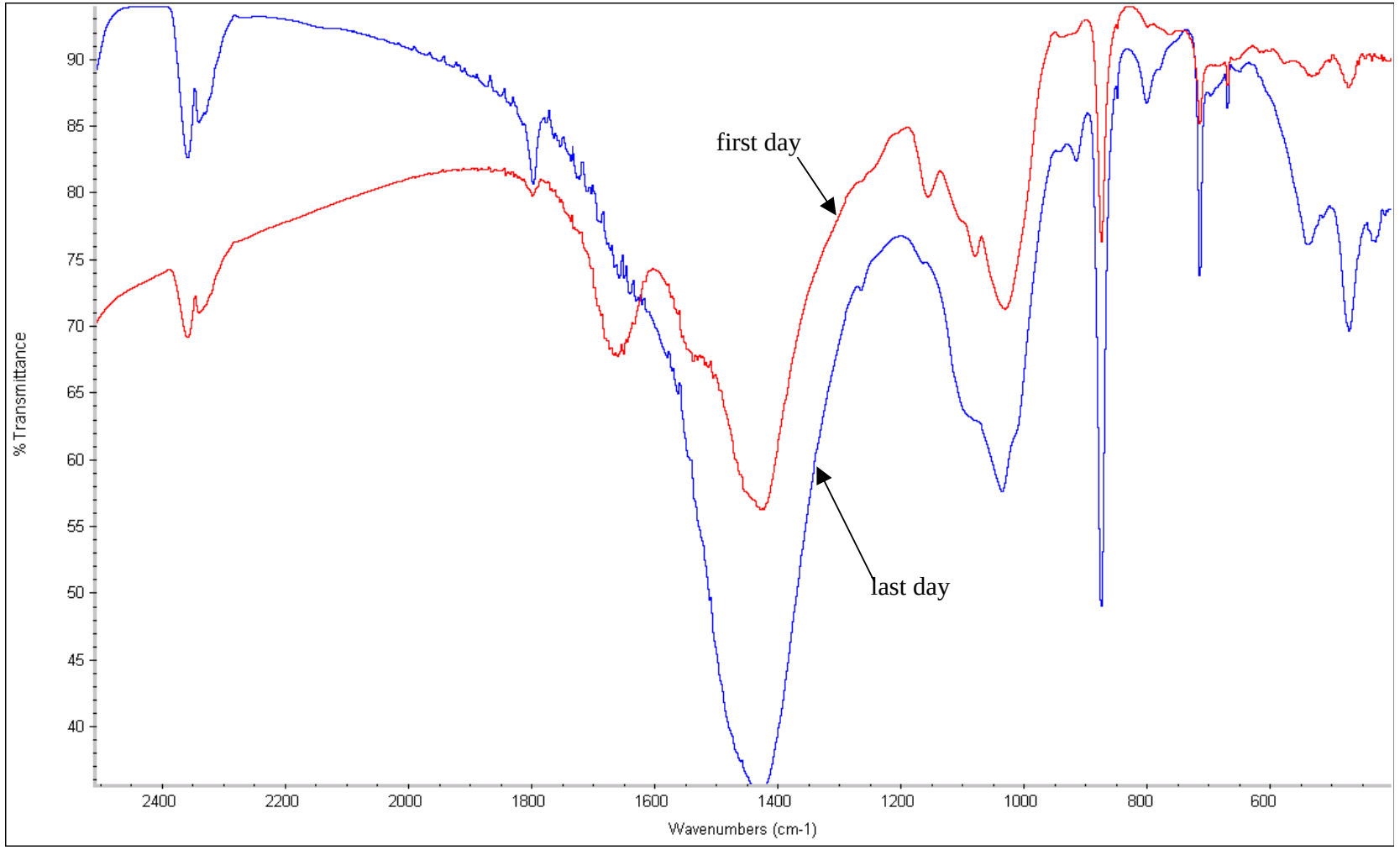

Figure 22:The infrared spectra of Oscillatoria showing correlation between the increase of its amounts by sun light exposure period and transmittance decreasing in the 2400-500 cm-1 region(First and last day).

Chapter 5

Conclusion and Recommendations

The use of microorganisms as indicators for water quality is widely used for exact determinations of water quality because each microorganism prefers its favorable conditions for abundance and growth. Many methods are widely used for monitoring water quality using bioindicators but most of them are expensive, special for some kinds of microorganism, slow so a need for rapid, inexpensive tool for water quality monitoring like FTIR.

This work includes two tests. **Qualitative test** was carried out to investigate the optimization and suitability of using FTIR in determination of each microorganisms found in water. Results showed that each microorganism has its special spectrum on FTIR because it has special sequence of functional groups in its membrane protein that make it differ from other microorganisms, Oscillatoria special spectrum was obtained by isolating it and preparing its standard.

Two different sites (sampling site I and II are both in Jericho) were studied for the presence of Oscillatoria (cyanobacteria) compared by Oscillatoria standard. The third sampling site was at Bethlehem. Results showed that Oscillatoria was present in Jericho while there was no Oscillatoria in sampling site III. The predominance of Oscillatoria in Jericho is due to hot, sunny days and warm, nutrient-rich waters which are Oscillatoria favorable conditions. The cyanobacteria bloom was most abundant during late summer and early autumn.

Oscillatoria as an example of cyanobacteria. It was dominant all over the year because of the mentioned reasons, its presence in water reflect high nutrient contents or Eutrophication of the water source. As a body of water becomes more eutrophic or polluted as the case in summer and early autumn, Oscillatoria increases in population.

The determination of water quality is an issue of very high importance due to the scarcity of water resources. Using bio indicators like cyanobacteria as an early warning of pollution or degradation in an ecosystem can help sustain there critical resources.

Cyanobacteria are a good example for these bio indicators because its presence in water is a direct indication of the water quality by:

1. Its presence is always accompanied by high nutrient level (Eutrophication).
2. Blooms containing even one species of toxic cyanobacteria will be poisonous and potentially dangerous.

Because there's no obvious way to tell if a particular bloom is toxic, samples have to be analyzed in a laboratory before a body of water can be declared safe.

Oscillatoria produces serious types of toxins: neurotoxins and hepatotoxins, neurotoxins can block the transmission of signals from neuron to neuron and neuron to muscle, while hepatotoxins cause bleeding in the liver. The threat is more to livestock then to humans

Exposure to Oscillatoria toxins through the ingestion of contaminated water or recreational contact is receiving increasing attention around the world as a public health concern. Recently the World Health Organization ,have made recommendations regarding 1 µg/L of microcystin for drinking water is the safe level guideline (Duncan et al.,2000).

The health risks posed by cyanobacterial toxins and the increasing anthropogenic eutrophication of potable and recreational waters have increased the need for rapid, sensitive method like FTIR to determine the presence of this type of cyanobacteria in water (James et al., 2001).

Many methods were used to detect water quality using bioindicator but FTIR is preferred because:

1. The spectra of almost any biological material can be obtained in a wide variety of environments, the amount of sample required is relatively small, The instrumentation is inexpensive, Interpretation of the spectra is not particularly difficult and can be learned easily.
2. Since different organisms differ in overall molecular composition, their FTIR spectra will also be different. The spectra can serve as spectroscopic fingerprints that enable highly accurate identification of microorganisms.

In the quantitative test . The optimization of FTIR in determination the amount of each type of microorganisms found in water was investigated.

The same water sample which was collected at 30/8/2006.This water sample was collected from sampling site I, this water sample showed Oscillatoria when was screened by the

compound microscope. The water sample was then exposed to sunlight in order to enhance Oscillatoria growth and proliferation. About 100ml from this sample for one week from 30/8/2006-6/9/2006 were filtered daily using whatman filter paper with 2.7um pores to isolate only oscillatoria filaments which have larger size than the pores of the filter paper. Increased, Oscillatoria filaments numbers were increased. All these water samples were also analyzed daily by FTIR for one week. Results showed that as Oscillatoria amount increased the transmittance on Oscillatoria spectrum decreased, this proves that FTIR can determine the quantity of Oscillatoria in each sample.

Based on the above and in order to protect our water resources, to protect health, to maintain sustainable development and to avoid losing long time and effort in water analysis with hard and difficult analytical methods the following are recommended:

- FTIR is highly recommended for biological water analysis for both qualitative and quantitative study of microorganisms.

- This method must be applied in the near future on various water resources in West Bank to study the distribution of Oscillatoria depending on our results (Oscillatoria spectrum).

- Study of Drinking water microorganisms content specially cyanobacteria and its toxins level in Jericho area must be done as soon as possible.

Oscillatoria detection based on FTIR technique is on a preliminary success for using this technique in water monitoring program and various fingerprints for the toxic algae and bacteria should be recorded and tested which are our suggestions for another work.

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Appendix

Monthly water samples were collected from sampling site I during one year from January 2006 till January 2007,all water samples were analyzed by FTIR in order to study its validity in identifying microorganisms types and amount that were found in water samples, FTIR results were as follow:

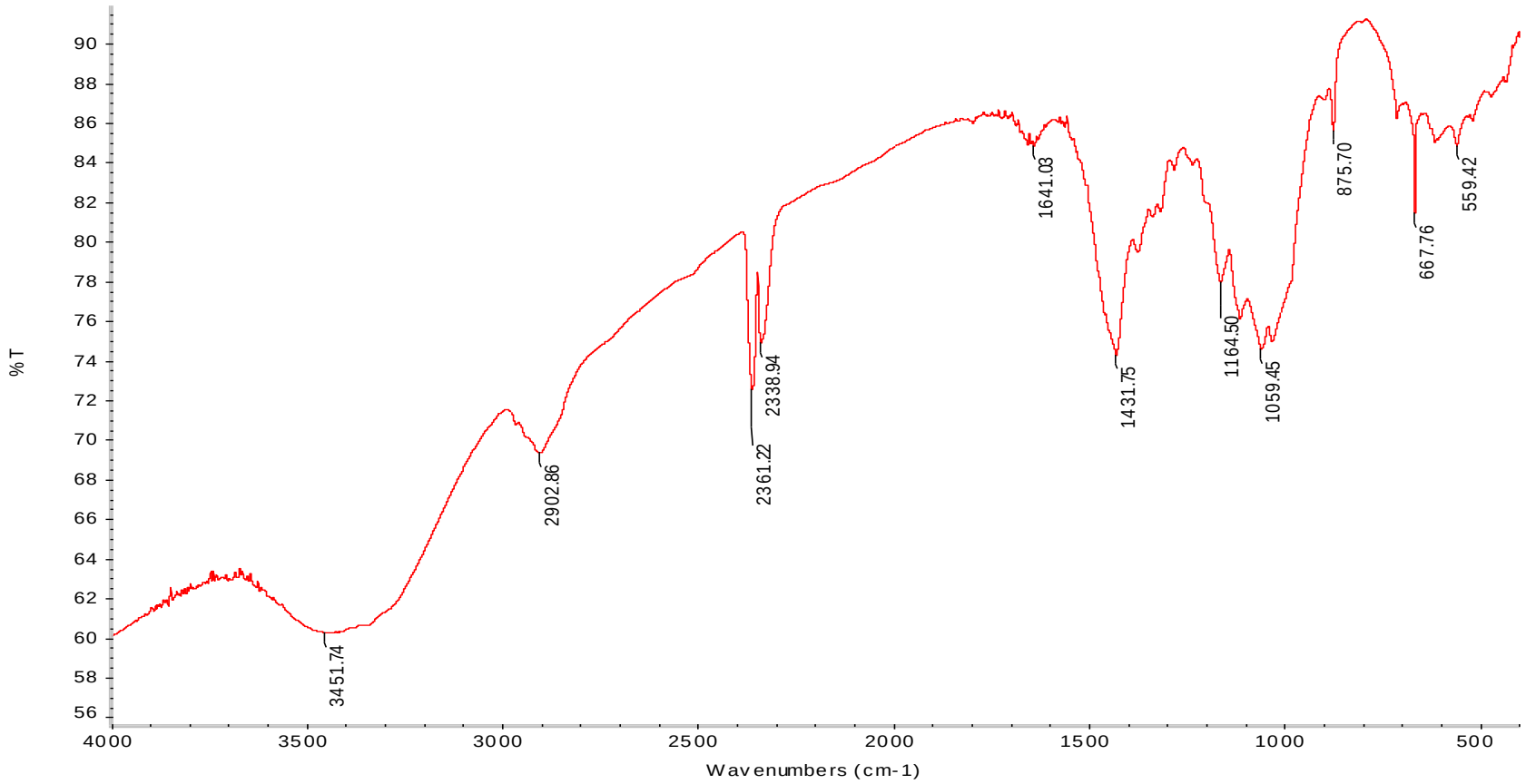

Figure 23:The infrared spectra of Oscillatoria for water sample which was collected from site I in January 2006 in the 4000-500 cm $^{-1}$ region.

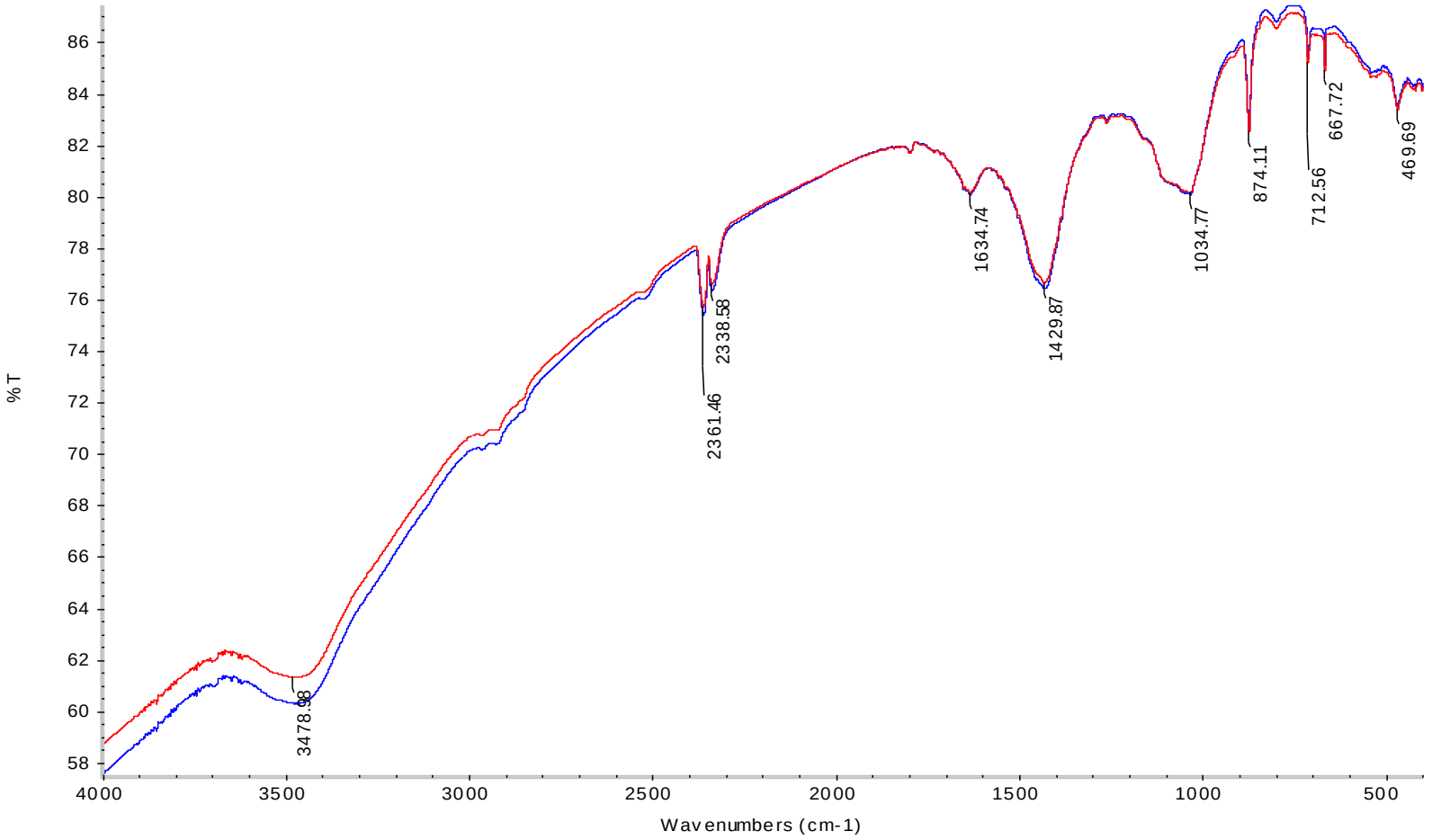

Figure 24: The infrared spectra of Oscillatoria for water sample which was collected from site I in February 2006 in the 4000-500 cm $^{-1}$ region.

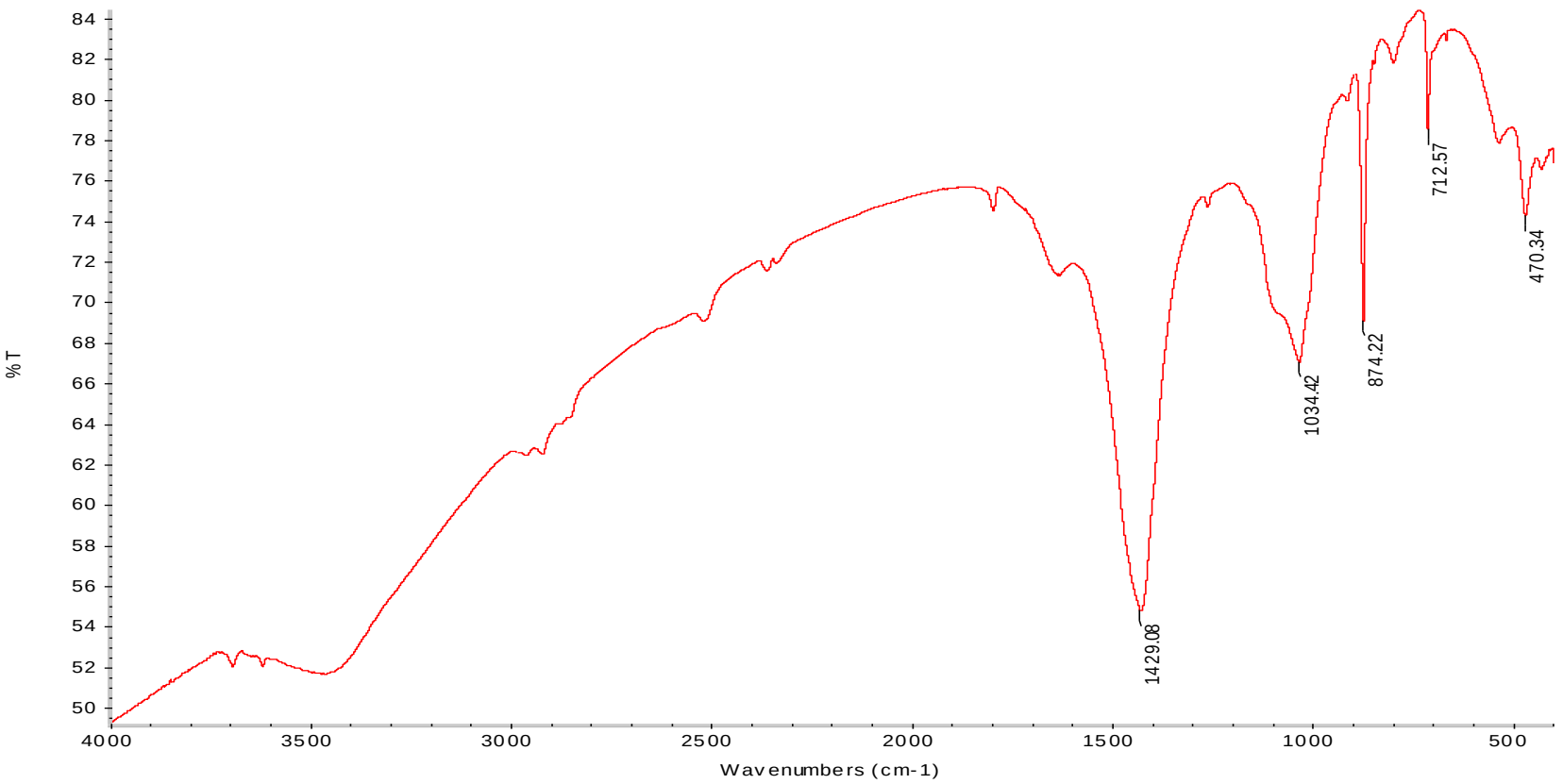

Figure 25: The infrared spectra of Oscillatoria for water sample which was collected from site I in April 2006 in the 4000-500 cm $^{-1}$ region.

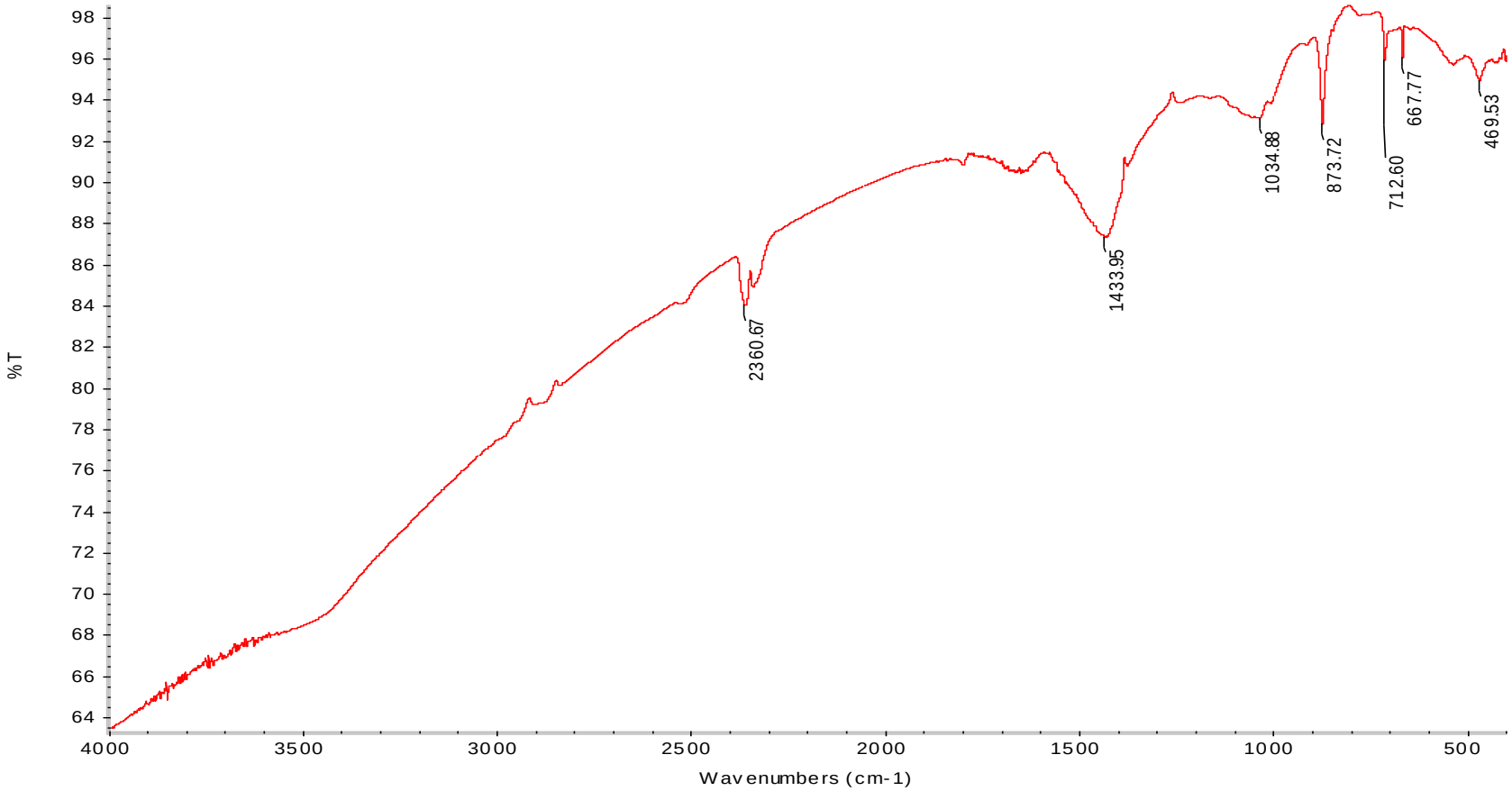

Figure 26: The infrared spectra of Oscillatoria for water sample which was collected from site I in May 2006 in the 4000-500 cm $^{-1}$ region.

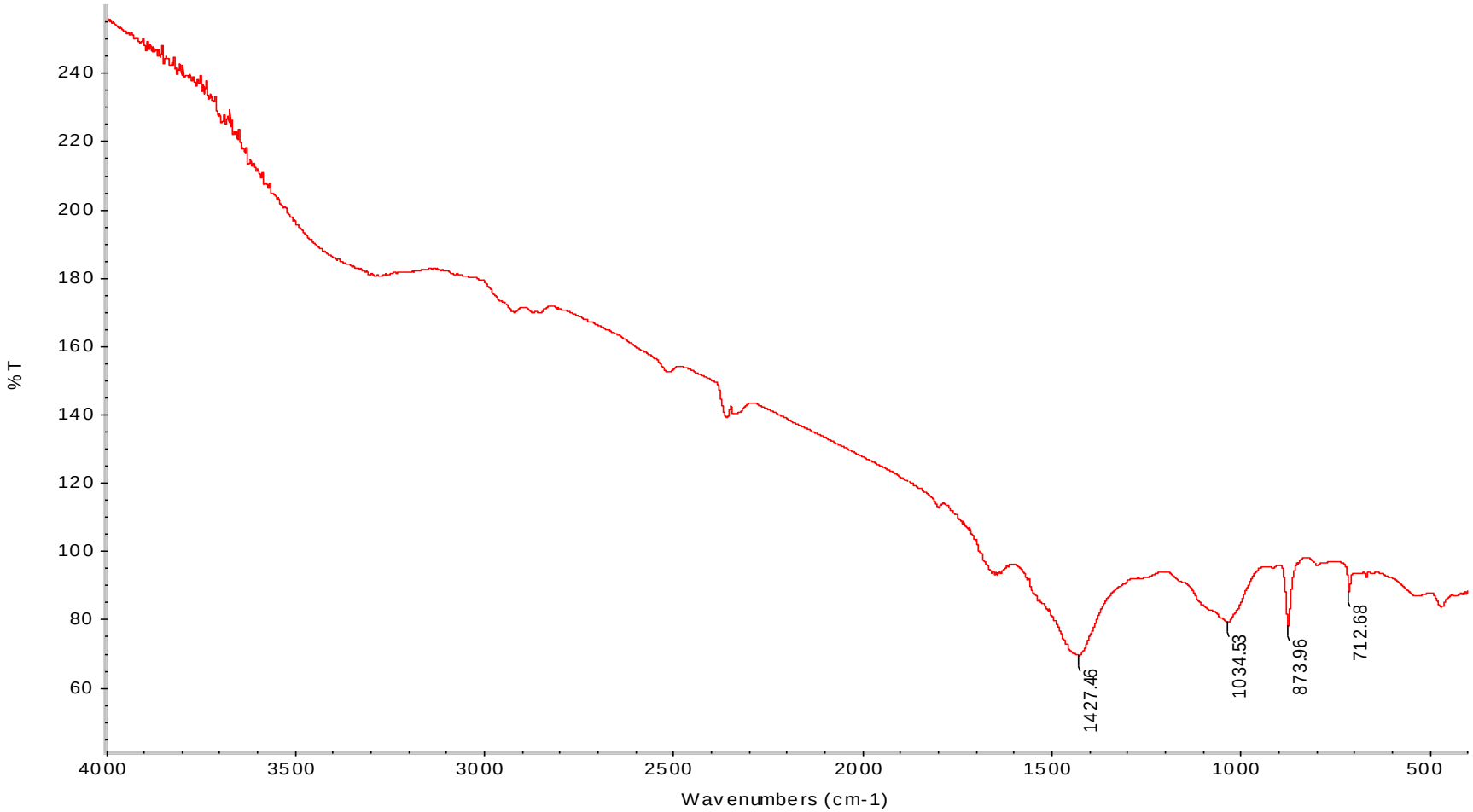

Figure 27 : The infrared spectra of Oscillatoria for water sample which was collected from site I in June 2006 in the 4000-500 cm $^{-1}$ region.

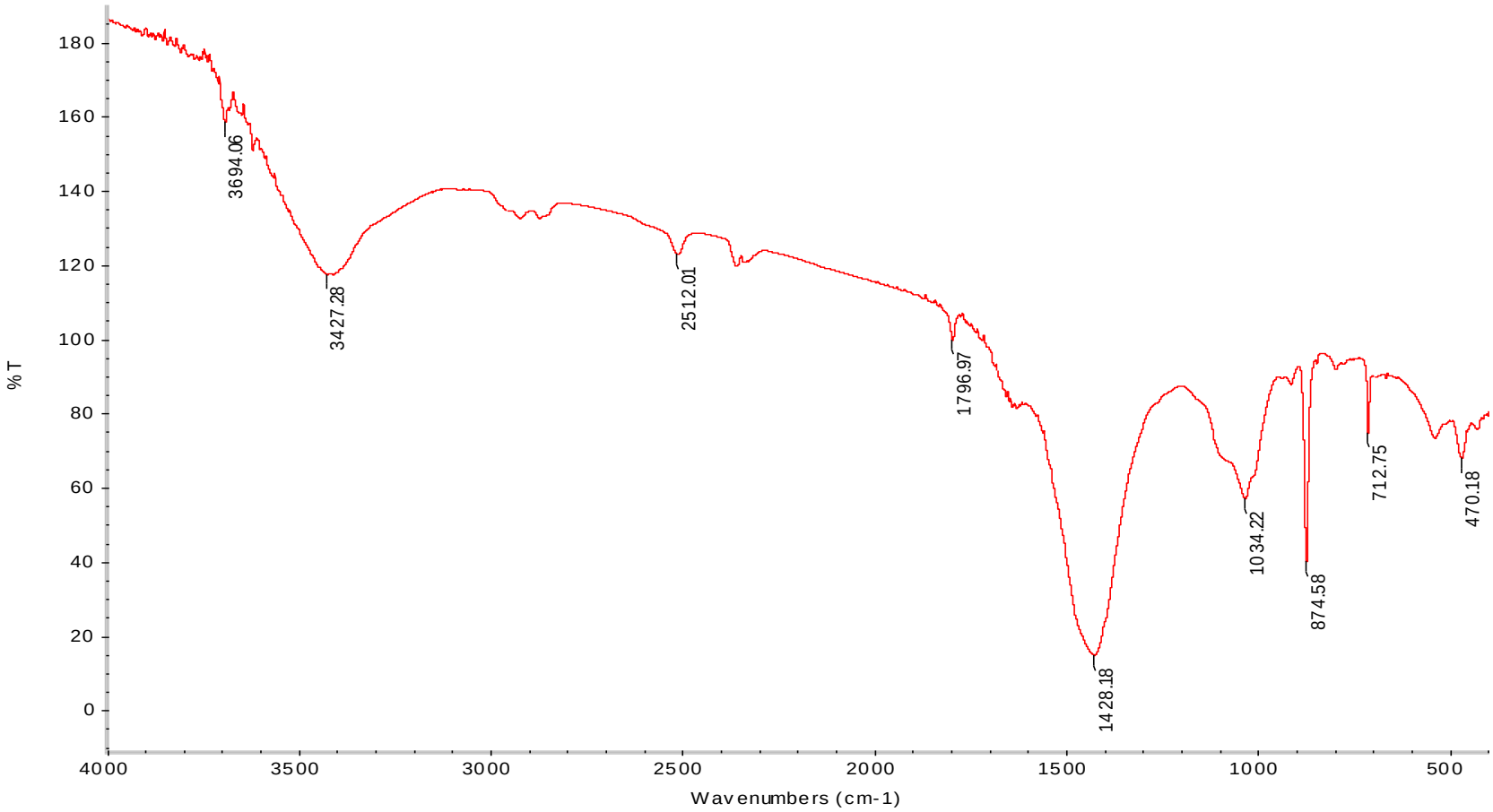

Figure 28: The infrared spectra of Oscillatoria for water sample which was collected from site I in July 2006 in the 4000-500 cm $^{-1}$ region.

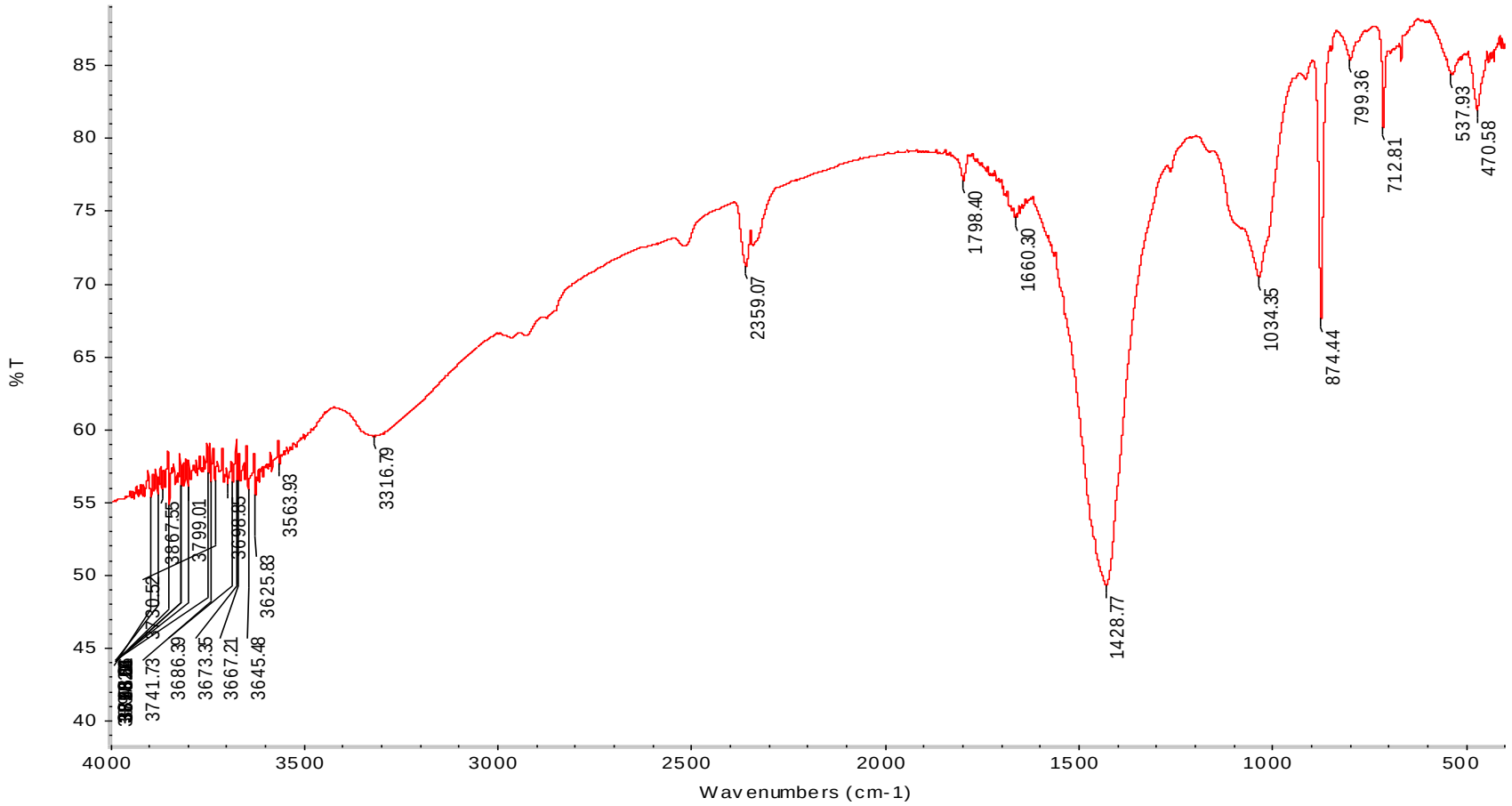

Figure 29: : The infrared spectra of Oscillatoria for water sample which was collected from site I in August 2006 in the 4000-500 cm $^{-1}$ region.

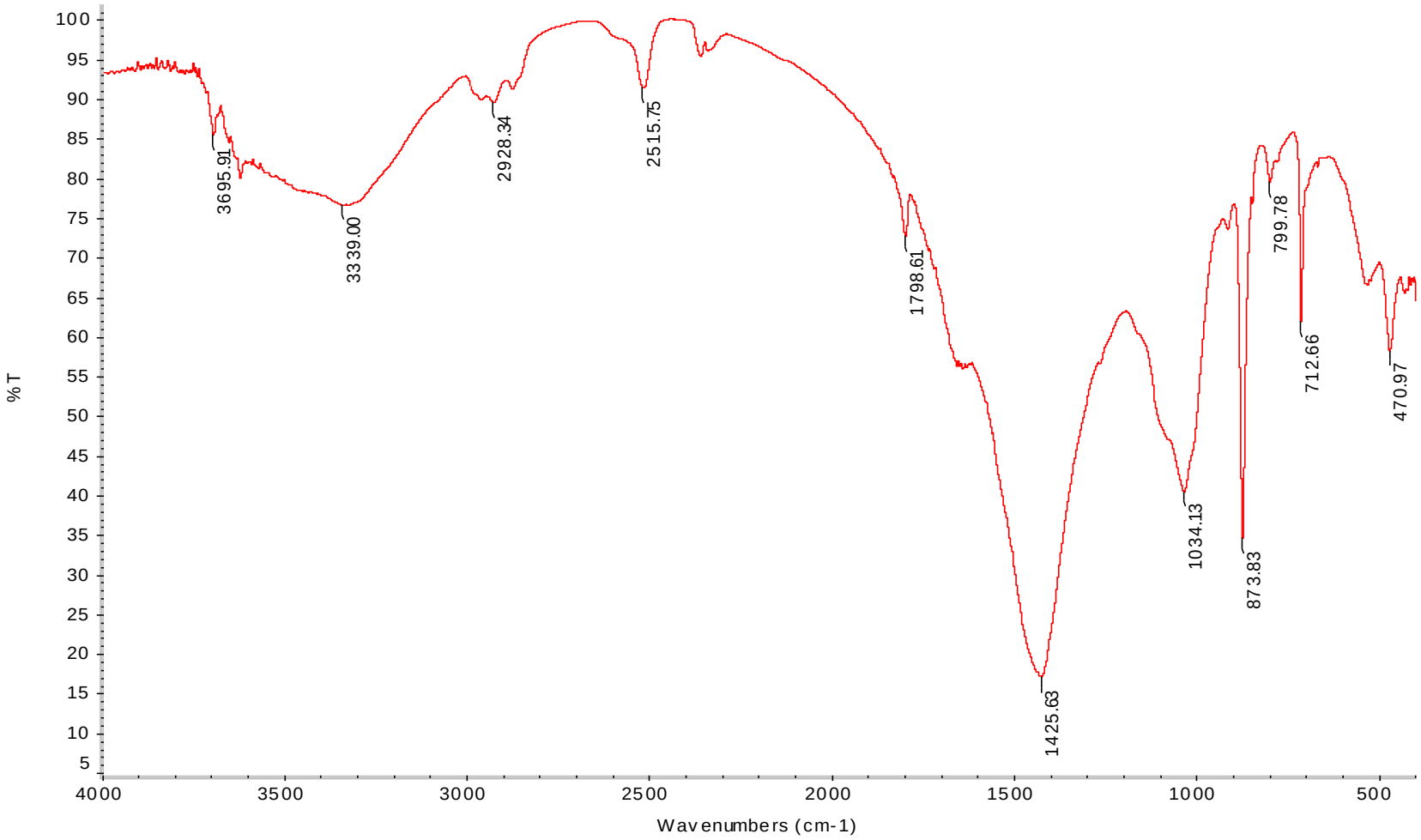

Figure 30: The infrared spectra of Oscillatoria for water sample which was collected from site I in September 2006 in the 4000-500 cm $^{-1}$ region.

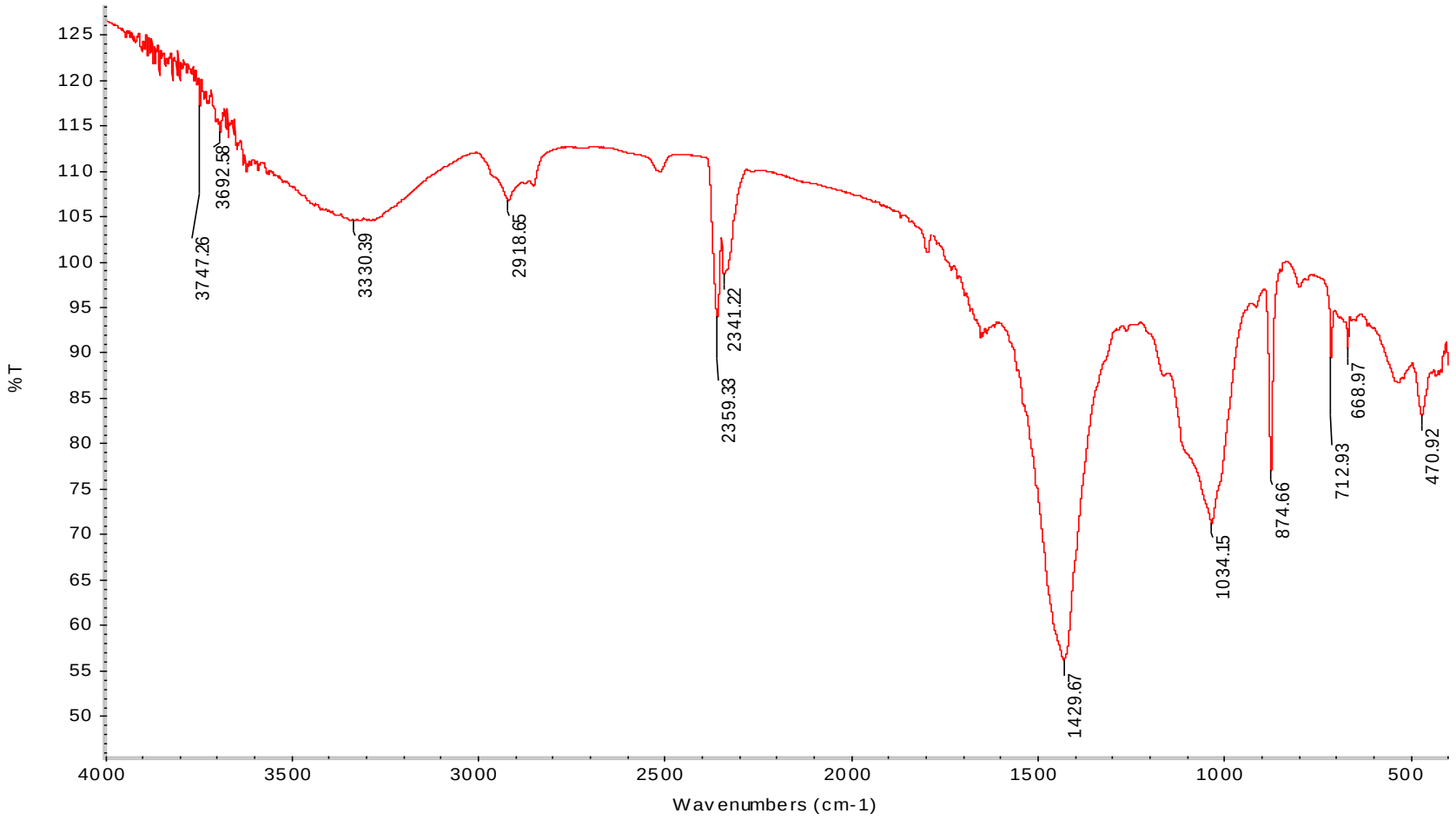

Figure 31: The infrared spectra of Oscillatoria for water sample which was collected from site I in november 2006 in the 4000-500 cm $^{-1}$ region.

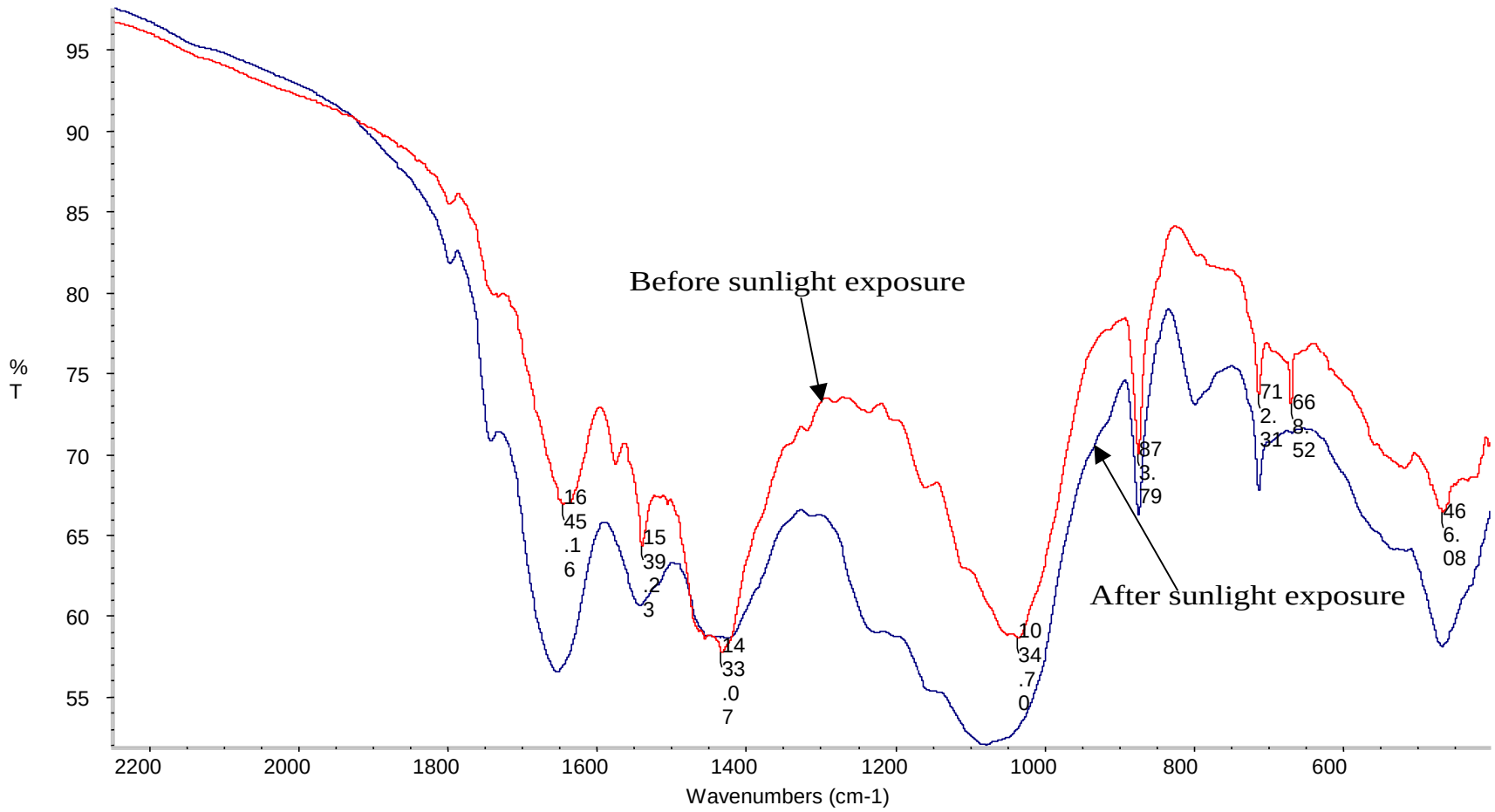

Figure 32:The infrared spectra of Oscillatoria which were found in water samples collected from site II in the 2200-500 cm^{-1} region, showing differences between Oscillatoria amount before and after exposure to sun light .

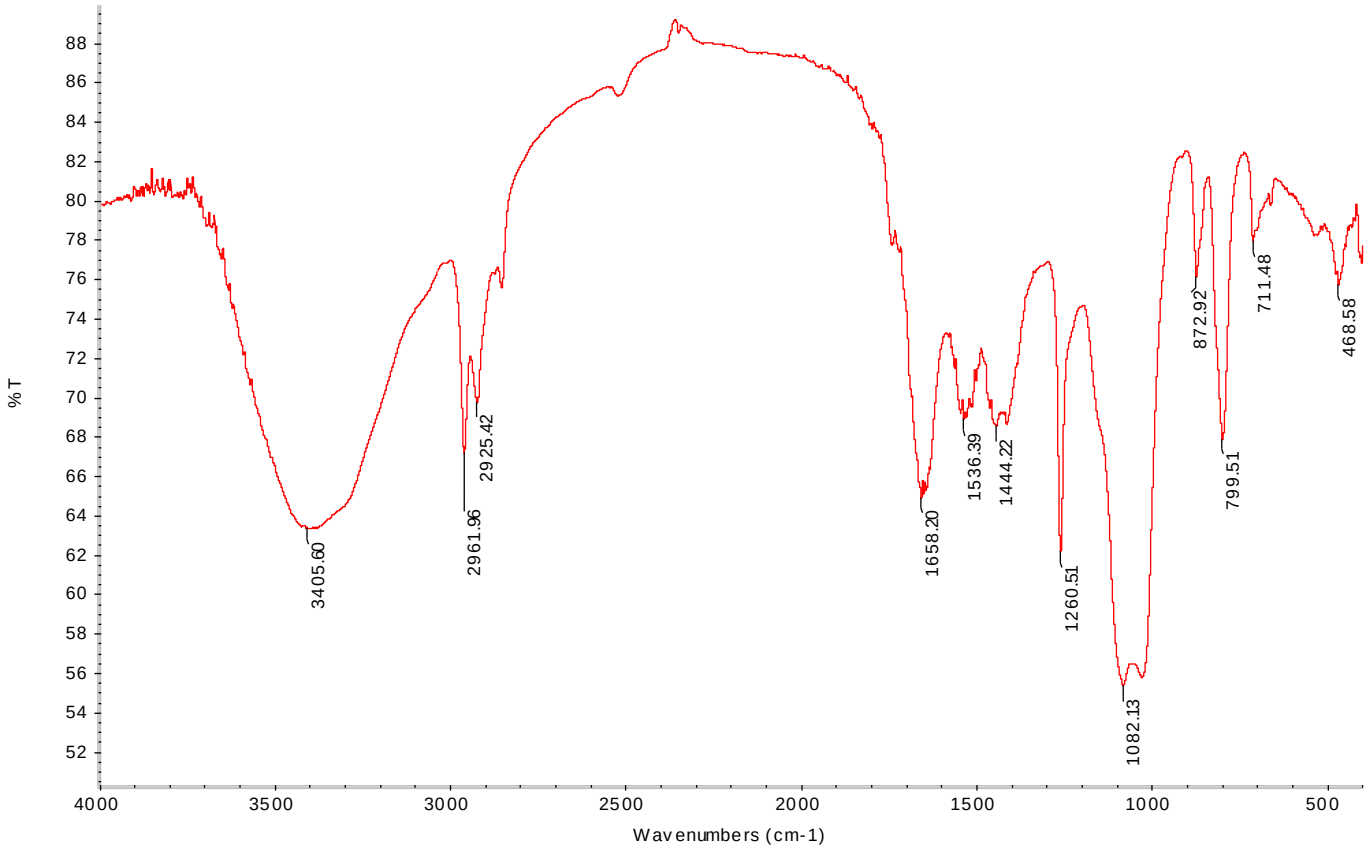

Figure 33: The infrared spectra of some imicroorganisms which were found in the water sample which was collected from site III in March 2007 in the 4000-500 cm $^{-1}$ region.

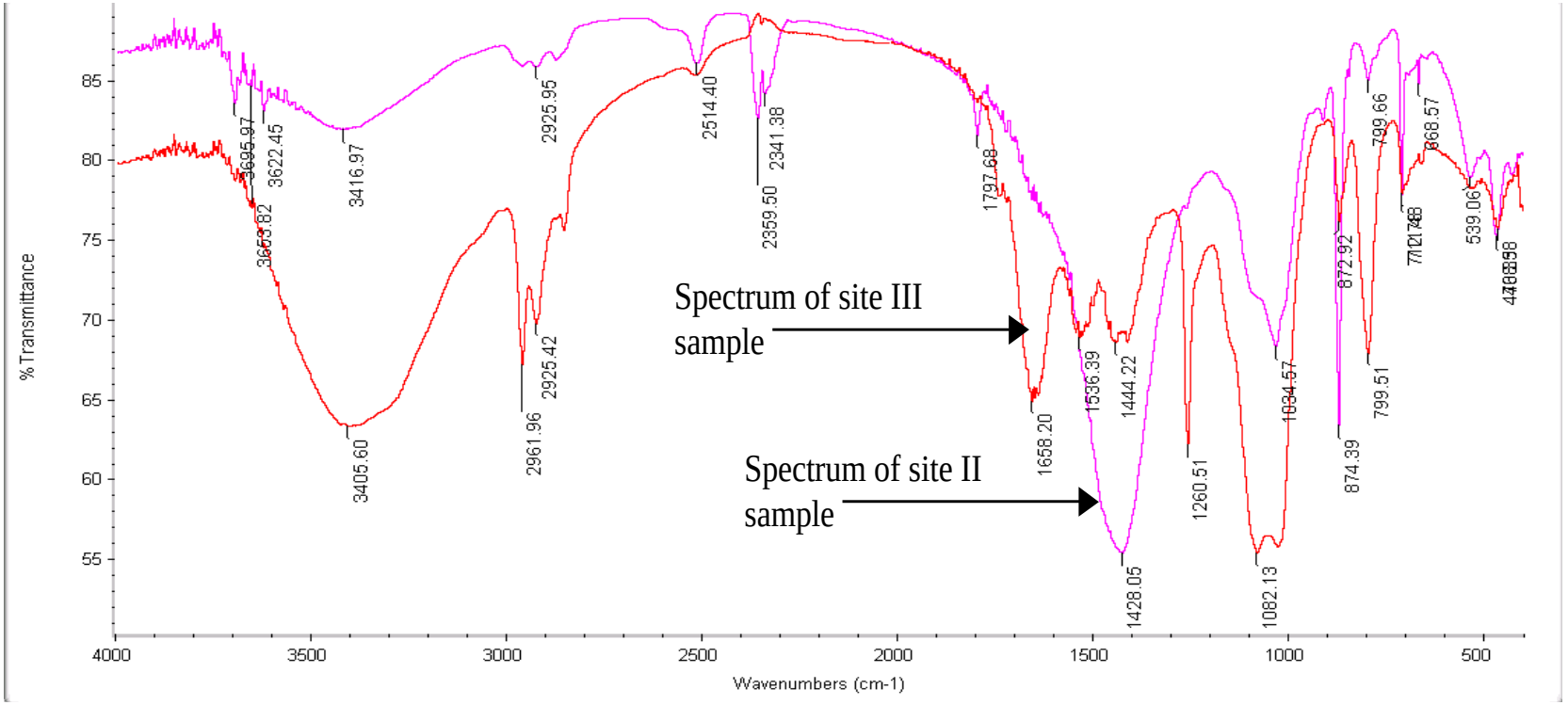

Figure 34: The infrared spectra of Oscillatoria which was collected from site II and the spectra of other microorganisms which were collected from site III showing two different fingerprints, in the 4000-500 cm^{-1} region.