HEAVY METAL-INDUCED STRUCTURAL AND FUNCTIONAL CHANGES IN CLINICAL AND ENVIRONMENTAL *ACINETOBACTER* ISOLATES

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

TUĞBA ÖZAKTAŞ

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY
IN
BIOLOGY

FEBRUARY 2015

Approval of the Thesis

HEAVY METAL-INDUCED STRUCTURAL AND FUNCTIONAL CHANGES IN CLINICAL AND ENVIRONMENTAL *ACINETOBACTER* ISOLATES

submitted by **TUĞBA ÖZAKTAŞ** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy in Biology Department, Middle East Technical University** by,

Prof. Dr. Gülbin Dural Ünver
Dean, Graduate School of **Natural and Applied Sciences**

Prof. Dr. Orhan Adalı
Head of Department, **Biology**

Prof. Dr. Ayşe Gül Gözen
Supervisor, **Biology Dept., METU**

Prof. Dr. Feride Severcan
Co-Supervisor, **Biology Dept., METU**

Examining Committee Members:

Prof. Dr. Mahinur Akkaya
Chemistry Dept., METU

Prof. Dr. Ayşe Gül Gözen
Biology Dept., METU

Prof. Dr. İrfan Kandemir
Biology Dept., Ankara University

Prof. Dr. Cumhur Çökmüş
Biology Dept., Ankara University

Assoc. Prof. Dr. Çağdaş Son
Biology Dept., METU

Date: 25.02.2015

I hereby declare that all information in this document has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conduct, I have fully cited and referenced all material and results that are not original to this work.

Name, Last name : Tuğba Özaktaş

Signature :

ABSTRACT

HEAVY METAL-INDUCED STRUCTURAL AND FUNCTIONAL CHANGES IN CLINICAL AND ENVIRONMENTAL *ACINETOBACTER* ISOLATES

ÖZAKTAŞ, Tuğba

Ph.D., Department of Biology

Supervisor: Prof. Dr. Ayşe Gül GÖZEN

Co-Supervisor: Prof. Dr. Feride SEVERCAN

February 2015, 98 pages

Heavy metal pollution is a threat resulting from increased anthropogenic activities. Cadmium (Cd), lead (Pb) and silver (Ag) are among the heavily used metals in different industrial areas. The accumulation of these hazardous substances in nature affects all organisms including human. Bacteria can tolerate these toxic heavy metals up to a degree by their intrinsic resistance mechanisms. Heavy metal resistance factors generally assist the spread of resistance to other toxic substances and antibiotics. *Acinetobacter* species are widely distributed opportunistic pathogens in nature. For assessing the molecular patterns of resistance as well as tolerance of *Acinetobacter* to heavy metals, an environmental and a clinical isolates were subjected to sub-lethal concentrations of Cd, Pb, and Ag. Extent of molecular changes was measured with ATR-FTIR spectroscopy by using alive intact bacterial cells. There were remarkable differences in molecular changes which manifest themselves as apparent resistance and tolerance strategies. These different strategies then lead to differences in physiologies between the isolates originating from two very different environments. This study showed that Pb was the most influential heavy metal on the cellular molecules; in turn it was the most tolerated one. Especially in environmental strain, Pb and Ag induced the extracellular

polysaccharide (EPS) synthesis. Furthermore, one of the noteworthy results of this study is that Pb, in environmental strain, caused formation of multiple strand polyribonucleotide aggregations. Interestingly, membrane dynamics were shaped by Cd and Pb in environmental isolate. In contrast clinical isolate did not exhibit measurable change in membrane dynamics. This study gave evidence on the adaptation to specific environments, by modulating the physiology of a bacterium arising from operating with different strategies. Measurable molecular changes than are attributable to the epigenetic potentials of bacteria which provides selections for modulation.

Keywords: Heavy metal resistance, MIC, *Acinetobacter*, ATR-FTIR spectroscopy, Cadmium, Lead, Silver

ÖZ

KLİNİK VE ÇEVRESEL *ACİNETOBACTER* İZOLATLARINDA AĞIR METAL İLE UYARILAN YAPISAL VE İŞLEVSEL DEĞİŞİKLİKLER

ÖZAKTAŞ, Tuğba
Doktora, Biyoloji Bölümü
Tez Yürütücüsü: Prof. Dr. Ayşe Gül GÖZEN
Ortak Tez Yürütücüsü: Prof. Dr. Feride SEVERCAN
Şubat 2015, 98 sayfa

Antropojenik etkilerin artışı ile oluşan ağır metal kirliliği büyük bir tehdit oluşturmaktadır. Kadmiyum (Cd), kurşun (Pb) ve gümüş (Ag) farklı endüstriyel alanlarda yoğun olarak kullanılan metaller arasındadır. Bu tehlikeli maddelerin doğada birikmesi, insan dahil tüm organizmaları etkilemektedir. Bakteriler bu ağır metalleri kendi doğal direnç mekanizmaları sayesinde bir dereceye kadar tolere edebilirler. Ağır metal direnç faktörleri genellikle diğer toksik maddelere ve antibiyotiklere olan direnç faktörlerinin de yayılmasını kolaylaştırırlar. *Acinetobacter* türleri doğada yaygın olarak bulunan fırsatçı patojenlerdir. Acinetobacter'in ağır metallere karşı olan direnç ve toleransının moleküler durumunu belirlemek için, çevresel ve klinik izolatları Cd, Pb ve Ag'nin subletal konsantrasyonlarına maruz bırakılmıştır. Zarar görmemiş canlı hücrelerdeki moleküler değişiklikler ATR-FTİR spektroskopisi ile ölçülmüştür. Belirlenen bu kayda değer değişimler bakterinin geliştirdiği direnç ve tolerans stratejisini oluşturmaktadır. Ortaya çıkan fizyolojik değişiklikler farklı çevrelerden gelen iki izolatın oluşturduğu farklı stratejiler kaynaklıdır. Bu çalışmaya göre, en çok tolere edilebilen ağır metal olan Pb, hücresel moleküller üzerindeki en etkili ağır metaldir. Çalışmada öne çıkan diğer sonuçlar ise

özellikle çevresel izolatta Pb ve Ag'nin hücre dışı polimerik maddelerin (EPS) sentezlenmesini tetiklemesi ve Pb'nin yine çevresel izolatta çift sarmallı ribonükleotidlerin çoğalmasına neden olmasıdır. Ayrıca çevresel izolatta Cd ve Pb kaynaklı hücre zarı dinamiklerinin yeniden şekillenmesi tespit edilmişken, bu tarz bir değişim klinik izolatta ölçülmemiştir. Bu çalışma, spesifik çevrelerdeki farklı stratejilerden kaynaklanan fizyolojik adaptasyonlara bir kanıt oluşturmaktadır. Ölçülebilen bu moleküler değişiklikler, bakterilerin değişimleri için seçilimlerine fırsat yaratan epigenetik potansiyallerine dayandırılabilir.

Anahtar Sözcükler: Ağır Metal Direnci, MİK, *Acinetobacter*, ATR-FTİR Spektroskopisi, Kadmiyum, Kurşun, Gümüş

To my parents and my brother

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to my supervisor Prof. Dr. Ayşe Gül Gözen for her advice, encouragement and supervision throughout this study.

I am also grateful to my co-supervisor Prof. Dr. Feride Severcan for her continuous help, suggestions and support in my work.

I would like to extend my thanks to the members of my thesis follow-up committee, Prof. Dr. Mahinur Akkaya and Assoc. Prof. Dr. Çağdaş Son for their constructive contributions during my work. I would also like to thank to Prof. Dr. Cumhur Çökmüş and Prof. Dr. İrfan Kandemir for their valuable suggestions.

I would like to thank to all my labmates and all members in Severcan's laboratory for their help and friendship.

I have special thanks to my beloved friends Burcu E. Tefon Öztürk, Sümeyra Gürkök, Aysun Özçelik, Tuba Çulcu, Fadime Kara Murdoch, Orhan Özcan, Gül İnan, Sıla Sungur and Elif Sevli for their invaluable help, support and friendship throughout this study.

I would like to give my deepest thanks to my father Hami Özaktaş, my mother Hülya Özaktaş, my brother Tunç Özaktaş and my family for their endless patience, support and understanding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Ag	Silver
ATR	Attenuated Total Reflectance
ATSDR	Agency for Toxic Substances and Disease Registry
AU	Arbitrary Units
Cd	Cadmium
CFU	Colony Forming Unit
Cr	Chromium
EPS	Extracellular Polysaccharides
FTIR	Fourier Transform Infrared
IR	Infrared
LPS	Lipopolysaccharide
MIC	Minimum Inhibitory Concentration
NA	Nutrient Agar
NB	Nutrient Broth
OD	Optical Density
Pb	Lead
SD	Standard Deviation
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Heavy metal pollution is a growing threat for environment and human health (Nithya et al., 2011; Huang and Liu, 2013). These toxic heavy metals are already present in the environment but they accumulate as a result of human activities. The major sources of this pollution are the coal, natural gas, paper, textile, cosmetic, food packaging, electroplating, and metal refining industries, mining and waste incineration plants (Bruins et al., 2000; Matlock et al., 2002; Wijnhoven et al., 2009; Huang and Liu, 2013; Naik and Dubey, 2013). Since heavy metals have long biological half-lives and they are non-biodegradable in the environment, they can be accumulated throughout the food chains and finally be hazard for human beings (Jiang and Fan, 2008; Martins et al., 2004).

Heavy metal accumulation in the environment and their toxic effects on the public health is regularly monitored by international organizations, such as the United States Agency for Toxic Substances and Disease Registry (ATSDR), the World Health Organization (WHO) (Jarup, 2003), the European Commission (Holm et al., 2002). According to Comprehensive Environmental Response, Compensation, and Liability Act 2013 Substance Priority List of ATSDR [http://www.atsdr.cdc.gov/SPL/index.html] lead (Pb) is the second and cadmium (Cd) is the seven in the top 10 most hazardous substances. Cd toxicity may reveal itself through syndromes and effects including renal dysfunction, hypertension, hepatic injury, lung damage and teratogenic effects (Hajialigol et al., 2006; Satarug et al., 2003; Alomar et al., 2010). Similarly, Pb is known to cause various types of serious health problems such as neurological and reproductive damages and cancer (Ahmedna et al., 2004; reviewed by Naik and Dubey, 2013). Although silver (Ag) has not been cited among the most hazardous heavy metals to public health yet, the increased usage of silver-based materials in various areas from health to electronics

is most likely to require caution in the near future due to its toxic impacts (Monteiro et al., 2009; Volker et al., 2013; Saulou et al., 2013). It is known that Ag has antimicrobial effect, also it was well documented that Ag ions are highly toxic to aquatic organisms (Bragg and Rainnie, 1974; Schreurs and Rosenberg, 1982; Ghandour et al., 1988; Eisler, 1996). In addition, argyria, impaired night vision, and abdominal pain can be seen in humans as a result of Ag toxicity (Rosenman et al., 1979; Rosenman et al, 1987; Simon, 2003; Braydich-Stolle et al., 2005).

The increase of heavy metals in the environment forces microbial communities to modify their compositions and metabolic capabilities for their sustainability (Guzzo and DuBow, 1994; Selvin et al., 2004). In other words heavy metals may act as driving force for the microbial evolution (Nithya et al., 2011). Microorganisms have adapted a variety of tolerance mechanisms to virtually all toxic metals. These mechanisms are generally plasmid-mediated and thus they easily spread throughout microbial communities (Rouch et al., 1995; Hoostal et al., 2008; Martinez et al., 2006). Heavy metal-resistant microorganisms may be useful as indicators of potential toxicity to other organisms (Jansen et al., 1994; Naik and Dubey, 2013).

It is already known that Cd and Pb are highly toxic for bacteria even at low concentrations (Nies, 1999; Trajanovska et al., 1997). Likewise, high concentrations of both nonessential and essential metals are lethal to bacteria via blocking functional groups of important molecules (Bruins et al., 2000). Specifically, it is known that Pb damages structures of DNA, protein and lipid, and also replaces essential ions in enzymes (Nies, 1999; Roane, 1999; Asmub et al., 2000; Hartwig et al., 2002). When Cd and Ag ions enter the cell, they easily interact with thiol (sulfhydryl) groups of proteins and inhibit the enzymes and eventually cellular metabolism is disrupted (Nies, 1992; Lebrun et al., 1994; Bruins et al., 2000; Nies, 1999; Hassen et al., 1998; Kim et al., 1998; Wang et al., 2010a). Also it was shown that Cd ions cause single-strand breaks in bacterial DNA (Trevors et al., 1986).

In this study, molecular changes in *Acinetobacter* species upon exposure to Pb, Cd, and Ag were investigated. The members of *Acinetobacter* genus are aerobic gram-negative rods. They are oxidase-negative, catalase-positive, non-motile, non-fermentative, capsulated, and ubiquitous bacteria (Mujumdar et al., 2014; de Breij et al., 2010; Euzeby, 1997). *Acinetobacter* species can be found in both soil and aquatic environments as a member of normal microbiota as well as opportunistic pathogen (Mujumdar et al., 2014; Pandey et. al., 2011). Since *Acinetobacter* species often appear as contaminant for drinking water (Bifulco et. al., 1989; Simoes et. al., 2008) and as participant of most important nosocomial infections (Rathinavelu et. al., 2003; Luna and Aruj, 2007; Giamarellou et. al., 2008; Gootz and Marra, 2008; Peleg et. al., 2008; Keen et. al., 2010; Tayabali et. al., 2012), they receive special attention in terms of public health concern. Specifically *A. haemolyticus* is an important human pathogen which causes the upper respiratory tract infections (Mujumdar et al., 2014), endocarditis (Martinez et al., 1995) and bloody diarrhea (Grotiuz et al., 2006). Moreover, *Acinetobacter* infections are not only limited to human clinical cases (Joly-Guillou, 2005; Ong et. al., 2009; Regalado et. al., 2009; Falagas et. al., 2007; Hu and Robinson, 2010; Sengstock et. al., 2010; Moreira Silva et. al., 2012; Ozaki et. al., 2009); they are also known as an important fish pathogen (Mujumdar et al., 2014; Pandey et. al., 2011). Furthermore, certain strains are used for biotechnological applications such as bioremediation of environmental toxins and bioengineering of enzymes in recent years (Luckarift et. al., 2011; Abdel-El-Haleem, 2003; Singh et. al., 2011; Jung et. al., 2011; Zhao et. al., 2011; Tayabali et. al., 2012).

In order to assess molecular changes in *Acinetobacter* species upon exposure to Pb, Cd, and Ag, ATR-FTIR spectroscopy was used in measurements. The principle of Fourier Transform Infrared (FTIR) spectroscopy is based on the absorption of the IR radiation. Chemical bonds in most molecules vibrate in different modes (Fig. 1). The energy of these molecular vibrations can be detected in the infrared region of electromagnetic spectrum (Haris and Severcan, 1999; Marcelli et al., 2012). Since certain types of covalent bonds and their modifications can be localized by specific

absorption peaks (Nichols et. al., 1985), IR spectrum provides detailed information about all biochemical components of cells, that is, proteins, lipids, polysaccharides and nucleic acids (Nichols et. al., 1985; Chittock et. al., 1999). Furthermore, FTIR spectroscopy can detect minute changes in molecular structure (Haris and Severcan, 1999). Thus FTIR spectroscopy has been successfully used to obtain information dealing with conformational changes in biomolecules as well as the information on quantitative changes (Naumann, 1984; Haris and Severcan, 1999). Changes in biochemical compositions of the intact microbial cells can also be analyzed by using FTIR spectroscopy (Naumann, 1984; Nichols et. al., 1985; Lamprell et al., 2006; Feo et al., 2004). Because FTIR spectra are specific enough each species of bacteria even in a strain level (Dziuba et al., 2007), they can be used for identification as well as characterization of molecular compositions under different stress conditions (Alvarez-Ordonez and Prieto, 2010; Alvarez-Ordonez et al., 2011). The Attenuated Total Reflectance (ATR) technique provides the analysis of living cells (Barth, 2007). With this technique the composition of bacteria can be nondestructively characterized by forming thin layers from liquid or solid samples (Nichols et. al., 1985; Haris and Severcan, 1999; Wang et al., 2010b).

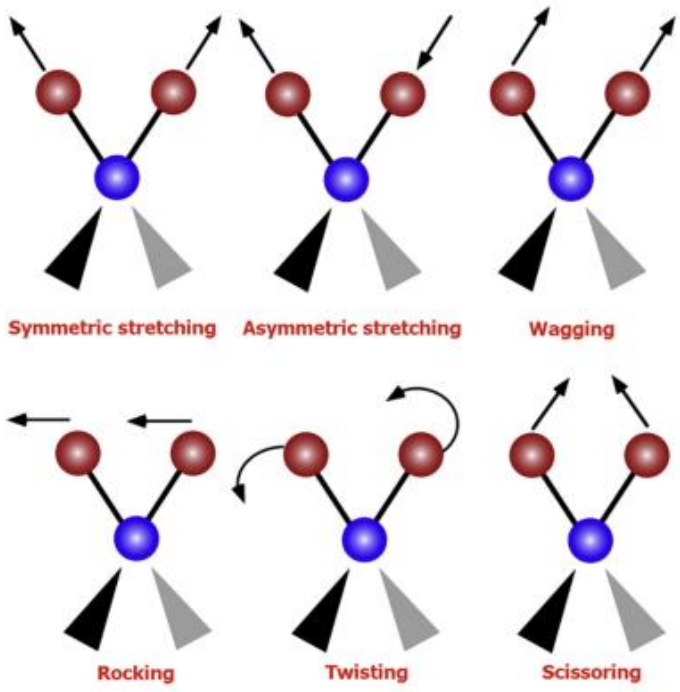

Figure 1. Simple illustrations of some vibrational modes of chemical bonds: two stretching modes and four different bending vibrations (Marcelli et al., 2012).

1.1 Aim and Scope

Our aim was to detect and measure the total molecular changes in an environmental and a clinical isolates of the same bacteria in response to heavy metal exposures. We hypothesized that the clinical and environmental bacteria should have different responses, if the environment that they are adapted to has marked influence on their genetics and in turn physiology.

Accumulated heavy metals are required to be cleaned-up from contaminated areas. Microorganisms are affected first from this type of environmental pollution, and they must develop some adaptations to survive in this kind of areas. Due to their specific ways to interact with heavy metals, bacteria are used in remediation processes of polluted environments. Although there are many studies related with metal resistance mechanisms, they are generally concentrated on specific biochemical mechanisms. In

present study, heavy metal-induced whole cell alterations were examined in more detail on bacteria isolated from natural and clinical environments. Two different *Acinetobacter* strains were chosen: environmental *Acinetobacter* sp. which is a freshwater fish derived isolate and clinical *Acinetobacter haemolyticus* ATCC 19002. To test our hypothesis, the two *Acinetobacter* were exposed to sub-inhibitory concentrations of three heavy metals (Cd, Pb, and Ag) and the molecular modifications in the whole bacterial cells were measured. This study contributes the field of microbial ecology by giving conclusive evidence on "bacteria adapted to different environments apply different strategies to cope with a given inhibitor".

CHAPTER 2

MATERIALS AND METHODS

2.1 Chemicals

The salts of heavy metals used in this study, $CdCl_2$. $2.5H_2O$, Pb $(NO_3)_2$ and $AgNO_3$ were obtained from Sigma-Aldrich. Stock solutions of heavy metals were prepared as 50 mg/ml. Working concentrations were started from 1 mg/ml and decreased in two-fold series down to 1.95 μg/ml (1000, 500, 250, 125, 62.50, 31.25, 15.63, 7.81, 3.9, 1.95 μg/ml). When it was required, in between concentrations were also used for the metals to obtain MIC values.

2.2 Microorganisms and Culture Conditions

Acinetobacter sp., a fish mucus-dwelling bacteria, was isolated from Lake Mogan, Ankara, Turkey and its 16S rRNA sequence can be reached in NCBI GenBank database under accession number JF421721 (Ozaktas et al., 2012). Well defined *Acinetobacter haemolyticus* ATCC 19002 was examined as reference bacteria for this research.

Bacteria were inoculated into nutrient broth (NB) medium consisting of (in g/L); peptone from meat (5 g) and meat extract (3 g) followed by overnight incubation at 200 rpm and 28°C. To determine the concentration of cells, the culture was serially diluted, then subsequently plated on nutrient agar (NA) and colony forming units (CFUs) were counted. For each culture optical density (OD) were also measured at 600 nm. The working concentrations of bacteria were set at 0.5 at OD_{600} which corresponded to10^9 CFU/ml.

2.3 Heavy Metal Resistance

Heavy metal resistance was determined by the broth dilution method. Minimum inhibitory concentration (MIC) is the lowest concentration leading to bacterial growth inhibition. After incubation for 48-72 h at 28°C, the MIC values of each tested heavy metal for two strains of *Acinetobacter* were determined. After that sub-inhibitory concentrations of each metal were experimented (Table 1). This values were the highest concentration of tested heavy metals which provide growth of bacteria upon 48 h incubation. All experiments were carried out in triplicates.

Table 1. Sub-inhibitory concentrations of *Acinetobacter* strains after 48 hours incubation time

Bacteria	**Tested Heavy Metals (µg/ml)**		
	Cd	**Pb**	**Ag**
Environmental *Acinetobacter* sp.	7.81	600	15.63
A. haemolyticus ATCC 19002	80	900	15.63

2.4 Sample Preparation for FTIR Spectroscopy Measurements

In order to find out the molecular changes in the metal exposed bacterial cells, a PerkinElmer Spectrum 100 FTIR spectrometer (Perkin-Elmer Inc., Norwalk, CT, USA) equipped with a Universal ATR accessory was used. Bacterial cells were grown with and without the metals (for metal-treated and control groups; respectively) for ATR-FTIR spectroscopy measurements. Bacterial cells were collected by centrifugation (10,000 X *g* for 10 min) (Schuster et al., 1999; Quilès et al., 2010; Kardas et al., 2014) and adjusted to working concentration mentioned above. The supernatant was decanted and pellets were dissolved in 15 µl sterile deionized water.

2.5 ATR-FTIR Spectroscopy Analysis

Infrared spectra were obtained between 4000-650 cm^{-1} region at 22 ± 1°C in an air conditioned room. A total of 100 scans were taken at a resolution of 4 cm^{-1}. Collection of spectra and processing of data were carried out using the Perkin-Elmer Spectrum 5.0.1 software. The background spectrum of air was substracted automatically. Totally 5 µl of bacterial suspension was placed on to the diamond/ZnSe crystal plate by sequential applications while drying with N_2 gas. Three separate bacterial suspensions of each sample were scanned. The average spectrum of this triplicate was used for further spectral and statistical analysis. Eventually, 10 spectra from these replicates were recorded for each group (control and metal-treated groups) of bacteria. Savitzky-Golay smooth function (at 9 points) was carried out to minimize of the noise. Band positions, band areas and bandwidths were determined after smoothing step: The wavenumber at the centers of the peaks were used for band position measurements. Besides smoothing, baseline correction was additionally required to calculate band areas. Also bandwidths were measured by the width of 0.75 X height of the peaks. Baseline corrected and normalized average spectrum of the 10 spectra was used for visual demonstration.

The absorption peak of Pb $(NO_3)_2$ itself was subtracted from the spectra of Pb-treated bacteria. For accurate subtraction, spectra of the Pb solution was recorded in the same conditions with the sample. The overlapping spectra of Pb solution in corresponding values (600 and 900 µg/ml) were digitally subtracted from the spectrum of the Pb-treated bacteria. Difference spectra with a subtraction factor of "1" were obtained for just Pb-treated groups.

2.6 Statistical Analysis

The data which was obtained from analysis of ATR-FTIR spectra were expressed as mean ± standard deviation (SD). The significance of differences was analyzed by

One-way Anova with Tukey's Multiple Comparison test and the results of each group were compared with each other. The p values less than or equal to 0.05 were considered as statistically significant. Degrees of significance were expressed as $*p \leq 0.05$; $**p \leq 0.01$; $***p \leq 0.001$; $****p \leq 0.0001$. All statistical analyses were carried out by using GraphPad Prism 6.01 software.

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Microbial Resistance to Heavy Metals

Metals, even essential ones, at high concentration are toxic. Cd, Pb and Ag are nonessential heavy metals and have no known biological role for microorganisms (Bruins et al., 2000). According to our measurements, the order of tolerance for environmental *Acinetobacter* sp. and *A. haemolyticus* ATCC 19002 were; Cd < Ag < Pb and Ag < Cd < Pb; respectively (Fig. 2). Among the three, Pb appeared to be the most tolerated heavy metal in both bacteria. Similar results were also reported dealing with Pb and Cd resistances for *A. haemolyticus* (Zakaria et al., 2007). In the presence of the three metals the bacteria grew slower. This slowing down was also reported for other bacteria (McEntee et al., 1986; Mergeay, 1991). This type of adaptation period is said to be crucial for especially induction of DNA repair mechanisms (Rouch et al., 1995) and adjustment of cell physiology to restrict the distribution of toxic metal in the cell or to repair damaged components (Mitra et al., 1975; Pages et al., 2007).

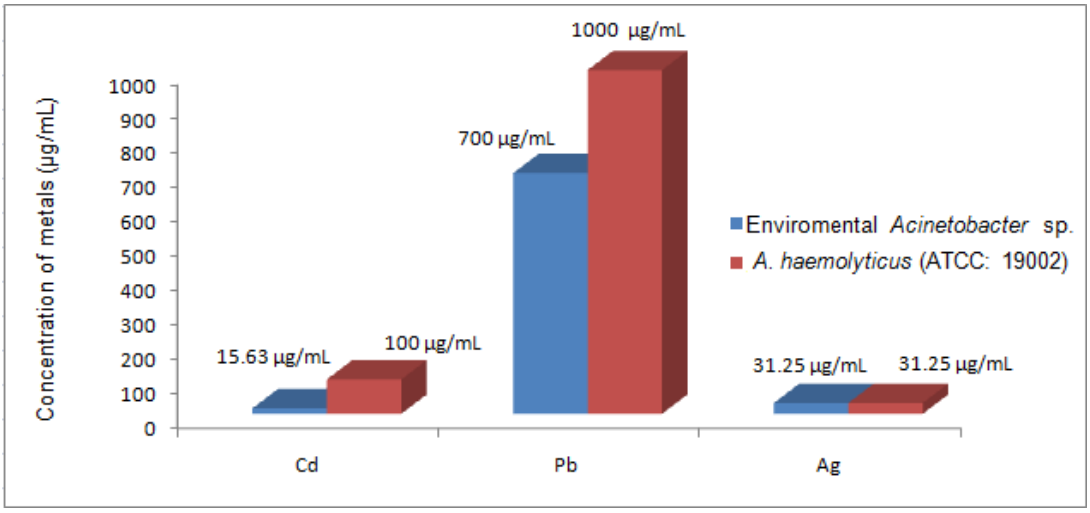

Figure 2. MIC of environmental *Acinetobacter* sp. and *A. haemolyticus* ATCC 19002 towards Cd, Pb, and Ag.

3.2 ATR-FTIR Analysis of Heavy Metal Exposed Bacterial Cells

ATR-FTIR spectra for the control (metal-free) and metal-treated cells were recorded to investigate the changes of cellular macromolecules in response to the heavy metal exposure. By analyzing the spectra, certain characteristic bands can be assigned to the main functional groups present in the bacterial cells. Table 2 represents the list of significant absorption peaks observed in the ATR-FTIR spectra following metal (Cd, Pb, and Ag) exposure in the two different *Acinetobacter* strains and related reports in literature. Also Fig. 3 shows the representative spectrum of control group in environmental *Acinetobacter* sp. with band numbers.

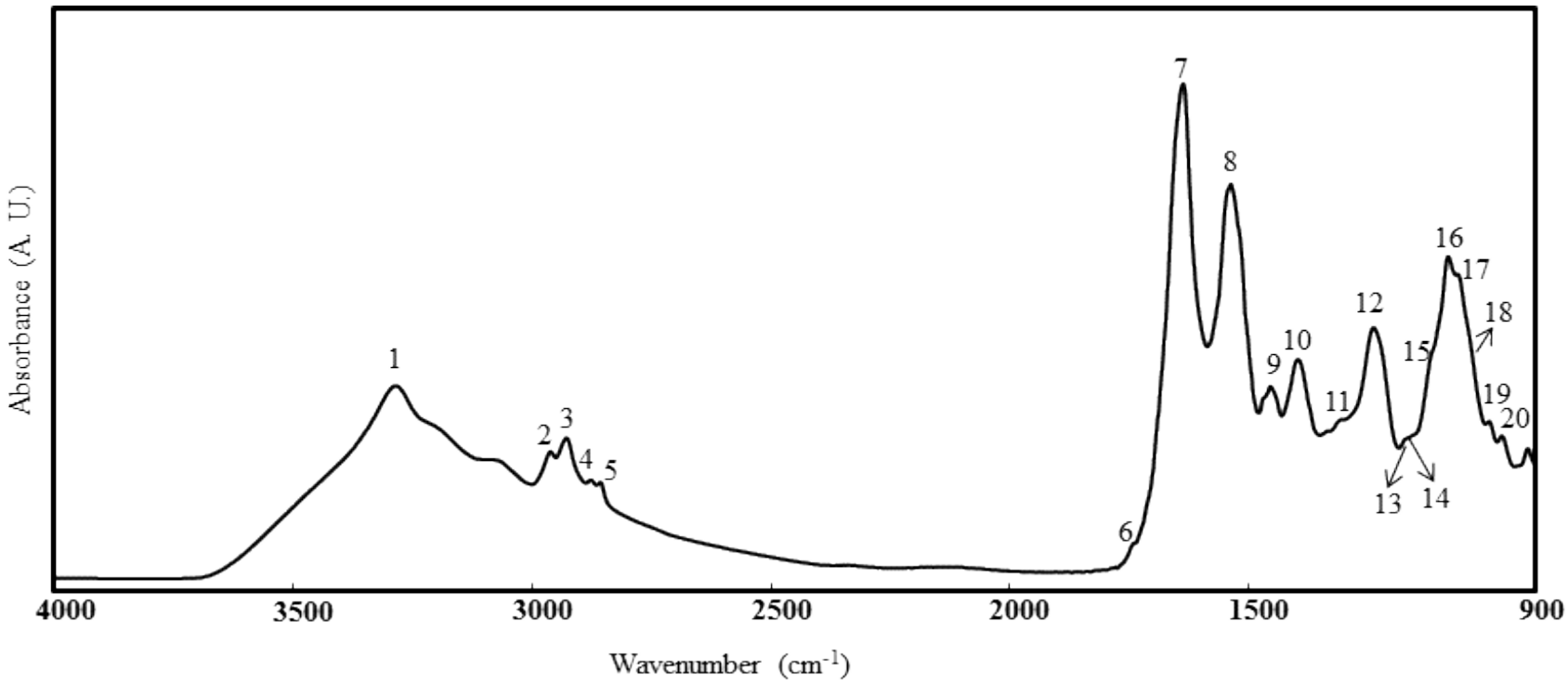

Figure 3. The representative IR spectrum of control environmental *Acinetobacter* sp. in the 4000-900 cm^{-1}.

Table 2. FTIR band assignments in the related literature

Band no	Band position (cm^{-1})	Spectral assignments	References
1	~ 3300 (Amide A)	ν(N-H) of amino groups & ν(O-H) of hydroxyl groups from proteins and polysaccharides	Pagnanelli et al., 2000; Barth & Zscherp, 2002; Gorgulu et al., 2007; Garip et al., 2009
2	2959	ν_{as} (C-H) of -CH_3 groups of fatty acids	Beech et al., 1999; Casal & Mantsch, 1984; Boyar & Severcan, 1997
3	2925	ν_{as} (CH_2) of lipids	Beech et al., 1999; Casal & Mantsch, 1984; Boyar & Severcan, 1997
4	2874	ν_s (CH_3) of mainly proteins with little contribution of lipids, carbohydrates and nucleic acids	Cakmak et al.,2006; Ozek et al., 2014
5	2852	ν_s (CH_2) of lipids	Schultz & Naumann, 1991; Casal & Mantsch, 1984; Boyar & Severcan, 1997
6	1741	ν(C=O) of triglycerides	Casal & Mantsch, 1984; Naumann, 1984; Severcan et al., 2005
7	~1650 (Amide I)	ν(C=O) of proteins	Barth & Zscherp, 2002; Haris & Severcan, 1999
8	~1550 (Amide II)	combination of δ(N-H) & ν(C-N) from proteins	Barth & Zscherp, 2002; Naumann, 2001; Ozek et al., 2014
9	1451	δ(CH_2) of lipids	Jiang et al., 2004; Cakmak et al.,2006

Table 2. (Cont.) FTIR band assignments in the related literature

Band no	Band position (cm^{-1})	Spectral assignments	References
10	1394	ν_s (COO^-) of amino acid side chains & free fatty acids	Naumann, 2001; Kardas et al., 2014
11	1400 to 1200 (Amide III)	combination of δ(N-H) & ν(C-N) from proteins/ components of proteins	Barth & Zscherp, 2002; Naumann, 2001; Kardas et al., 2014
12	1233	ν_{as} (PO_2) of mainly nucleic acids with little contribution of phospholipids	Naumann, 2001; Cakmak et al.,2006
13	1173	combination of ν(CO) & δ(COH) from polysaccharides & ν(PO) from phosphate groups	Sockalingum et al., 1997; Banyay et al., 2003
14	1156	sugar ring vibration from cell wall	Gao & Chorover, 2009; Sockalingum et al., 1997
15	1117	ν(C-O) of ribose	Liquier et al.,1991; Banyay et al., 2003
16	1082	ν_s(PO_2) of mainly nucleic acids with little contribution of phospholipids	Naumann, 2001; Garip et al., 2009; Kardas et al., 2014
17	1056	ν_s(C-O-C) & ν_s(P-O-C) of polysaccharides on capsule and peptidoglycan	Quiles et al., 2010; Kardas et al., 2014
18	1034	ν(CO) & ν(CC) of alcohols & carboxylic acids & δ(COH) of polysaccharides mainly on cell wall	Bouhedja et al., 1997; Huang & Liu, 2013
19	992	Ribose skeleton	Liquier et al.,1991; Quiles et al., 2010; Kardas et al., 2014
20	966	ν(CC) of DNA and RNA backbones	Cakmak et al.,2006; Garip et al., 2009

ν, stretching vibration; ν_s, symmetric stretching vibration; ν_{as}, asymmetric stretching vibration; δ, bending vibration

3.2.1 Changes in Cellular Components after Heavy Metal Exposure

The frequencies of the molecular vibration can be monitored using the absorption of IR light (Haris and Chapman, 1992; reviewed by Arrondo et al. 1993; Goormaghtigh et al. 1994; Siebert, 1995). The vibrational spectrum of biomolecules is directly influenced by intra- and intermolecular situations (Barth and Zscherp, 2002). Thus conformational changes (Garip et al., 2009; Ozek et al., 2014; Barth and Zscherp, 2002), conformational freedom and flexibility (Barth and Zscherp, 2002; Barth, 2007), and alterations in their concentrations (Kardas et al., 2014) can be deduced from the spectral parameters: band position, bandwidth and band area; respectively. In this study, the differences between control and heavy metal-treated groups provided knowledge about molecular changes under the influence of heavy metals in different bacteria. The significant differences between controls and treated groups were given in tables 3-5 and in figures 4-8 in terms of band position, band area and bandwidth for both *Acinetobacter* strain. Also these changes were shown in figures 9-14.

Table 3. The band frequencies with significant differences between control and heavy metal treated environmental *Acinetobacter* sp.

Band no	Frequency values (cm^{-1}) for environmental *Acinetobacter* sp. (n=10)									
	Control vs. 7.8 µg/ml Cadmium				Control vs. 600 µg/ml Lead			Control vs. 15.63 µg/ml Silver		
	Ctrl Mean ± SD	Cd Mean ± SD	% change	*p*-value	Pb Mean ± SD	% change	*p*-value	Ag Mean ± SD	% change	*p*-value
1	3263.53 ± 3.59	3264.07 ± 5.15	-0.02	ns	3258.17 ± 3.31	0.16	*	3264.81 ± 3.08	-0.04	ns
4	2874.24 ± 0.42	2874.58 ± 0.25	-0.01	ns	2874.94 ± 0.35	-0.02	****	2874.64 ± 0.19	-0.01	*
5	2852.61 ± 0.37	2851.85 ± 1.20	0.03	ns	2851.32 ± 0.48	0.05	**	2851.49 ± 1.00	0.04	*
9	1451.37 ± 0.29	1450.67 ± 0.54	0.05	**	1449.55 ± 0.47	0.13	****	1450.51 ± 0.29	0.06	***
10	1394.31 ± 0.50	1393.17 ± 0.95	0.08	*	1392.45 ± 0.99	0.13	***	1392.54 ± 0.84	0.13	***
11	1308.49 ± 0.98	1309.57 ± 2.60	-0.08	ns	1311.72 ± 0.81	-0.25	***	1311.20 ± 0.84	-0.21	**
12	1234.18 ± 0.77	1234.55 ± 1.56	-0.03	ns	1234.55 ± 1.02	-0.03	ns	1232.41 ± 1.02	0.14	**
14	NO	NO	-	-	1155.11 ± 1.42	-	-	1161.26 ± 2.37	-	-
15	1118.16 ± 0.17	1118.96 ± 0.4	-0.07	ns	1120.05 ± 1.82	-0.17	***	1118.92 ± 0.25	-0.07	ns
16	1082.70 ± 0.26	1082.12 ± 0.40	0.05	**	1081.34 ± 0.46	0.04	****	1081.97 ± 0.09	0.07	***
17	1056.46 ± 0.29	1055.51 ± 0.44	0.09	ns	1053.57 ± 2.57	0.27	***	1055.32 ± 0.16	0.18	ns
18	1034.15 ± 0.49	1032.27 ± 0.67	0.18	****	1032.22 ± 0.45	0.19	****	1032.45 ± 0.89	0.16	****
19	992.83 ± 0.20	992.62 ± 0.25	0.02	ns	978.88 ± 7.62	1.43	****	992.56 ± 0.25	0.03	ns
20	966.23 ± 0.27	966.45 ± 0.30	-0.02	ns	NO	-	-	966.53 ± 0.64	-0.03	ns

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, non-specific; NO, not observed
The "-" indicates increases and the "+" shows decreases when compared to control group values

Table 4. The band frequencies with significant differences between control and heavy metal treated *A. haemolyticus* ATCC 19002

Band no	Frequency values (cm^{-1}) for Acinetobacter haemolyticus ATCC 19002 (n=10)									
	Control vs. 80 µg/ml Cadmium				Control vs. 900 µg/ml Lead			Control vs. 15.63 µg/ml Silver		
	Ctrl Mean ± SD	Cd Mean ± SD	% change	*p*-value	Pb Mean ± SD	% change	*p*-value	Ag Mean ± SD	% change	*p*-value
1	3263.58 ± 3.19	3264.55 ± 1.86	-0.03	ns	3263.70 ± 3.22	0.00	ns	3271.95 ± 3.15	-0.26	****
4	2873.73 ± 0.49	2874.18 ± 0.50	-0.02	ns	2874.73 ± 0.47	-0.03	***	2874.84 ± 0.66	-0.04	***
7	1640.27 ± 1.68	1639.64 ± 0.53	0.04	ns	1636.97 ± 0.29	0.20	****	1639.21 ± 0.87	0.06	ns
8	1536.53 ± 2.00	1535.28 ± 0.91	0.08	ns	1536.62 ± 0.97	-0.01	ns	1538.17 ± 1.18	-0.11	*
9	1450.61 ± 1.66	1448.35 ± 0.38	0.16	*	1448.71 ± 0.55	0.13	**	1449.78 ± 1.74	0.06	ns
10	1392.79 ± 1.05	1394.89 ± 0.97	-0.15	****	1391.95 ± 0.62	0.06	ns	1393.15 ± 0.94	-0.03	ns
11	1313.17 ± 1.31	1302.97 ± 1.55	0.78	****	1312.16 ± 1.83	0.08	ns	1301.74 ± 3.09	0.88	****
12	1232.46 ± 1.33	1232.23 ± 0.65	0.02	ns	1231.96 ± 0.52	0.04	ns	1233.93 ± 0.96	-0.12	**
13	1174.44 ± 0.85	1173.42 ± 0.10	0.09	**	1173.84 ± 0.13	0.05	ns	1174.80 ± 1.00	-0.03	ns
14	1156.78 ± 0.90	1156.98 ± 0.31	-0.02	ns	1156.14 ± 0.26	0.06	*	1157.14 ± 0.28	-0.03	ns
15	1116.19 ± 1.07	1115.31 ± 0.58	0.08	ns	1110.55 ± 1.54	0.51	****	1113.21 ± 0.46	0.27	****
16	1082.37 ± 0.29	1082.19 ± 0.31	0.02	ns	1081.60 ± 0.31	0.07	****	1082.03 ± 0.29	0.03	ns
17	1057.15 ± 0.35	1056.32 ± 0.20	0.08	****	1053.62 ± 0.42	0.34	****	1056.18 ± 0.37	0.09	****
18	1031.02 ± 0.77	1029.00 ± 0.57	0.20	****	1028.60 ± 0.55	0.24	****	1028.79 ± 0.43	0.22	****
19	991.98 ± 0.22	992.14 ± 0.12	-0.02	ns	991.04 ± 0.26	0.09	****	992.02 ± 0.71	0.00	ns
20	966.04 ± 0.59	965.52 ± 0.23	0.05	ns	968.01 ± 1.43	-0.20	****	965.94 ± 0.27	0.01	ns

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, non-specific
The "-" indicates increases and the "+" shows decreases when compared to control group values

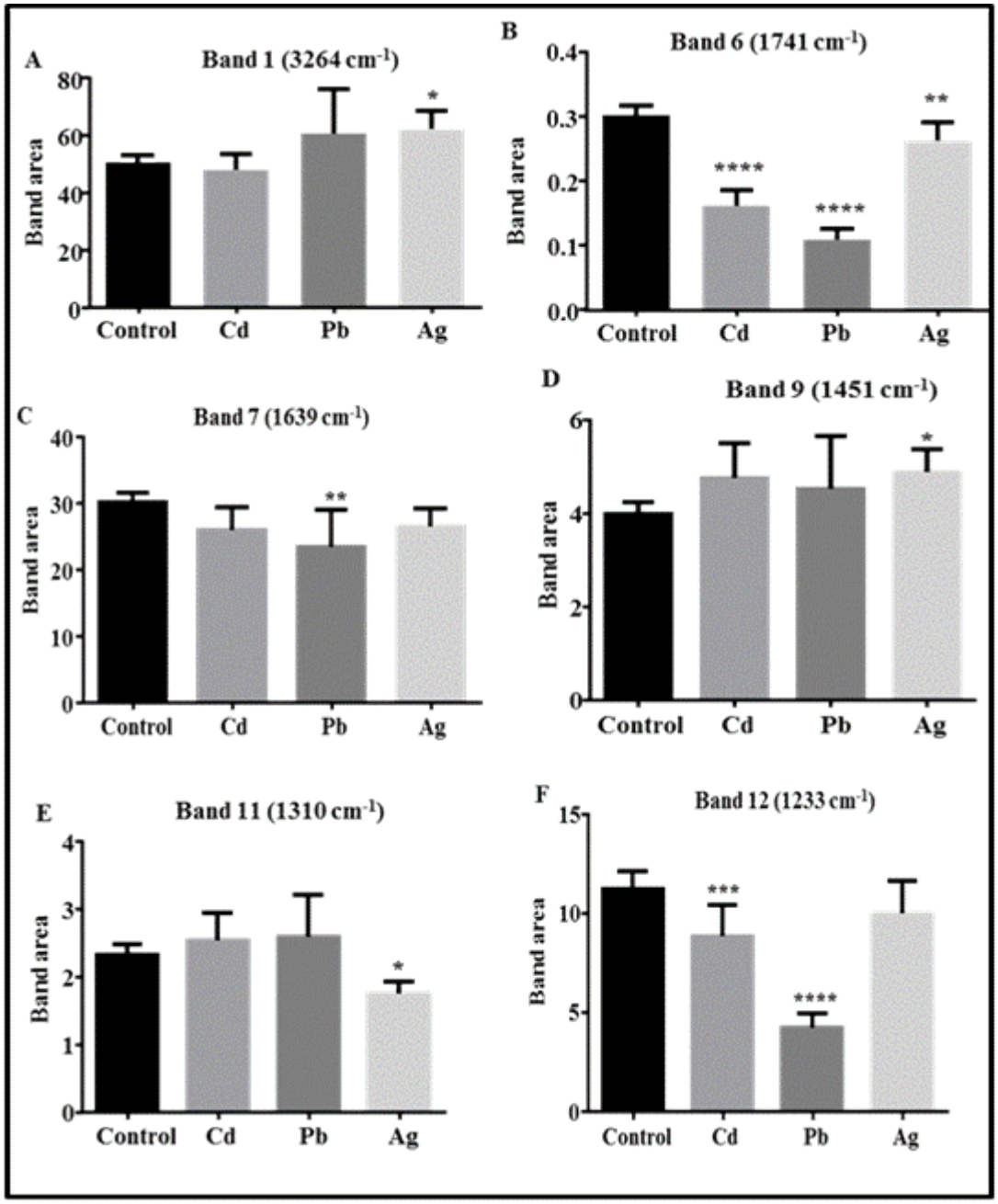

Figure 4. Bar diagram representing the concentration information obtained from the band areas of control and heavy metal treated environmental *Acinetobacter* sp. in the 4000-1200 cm^{-1} region. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ represent the degree of significance, which is against control for each heavy metal treated groups.

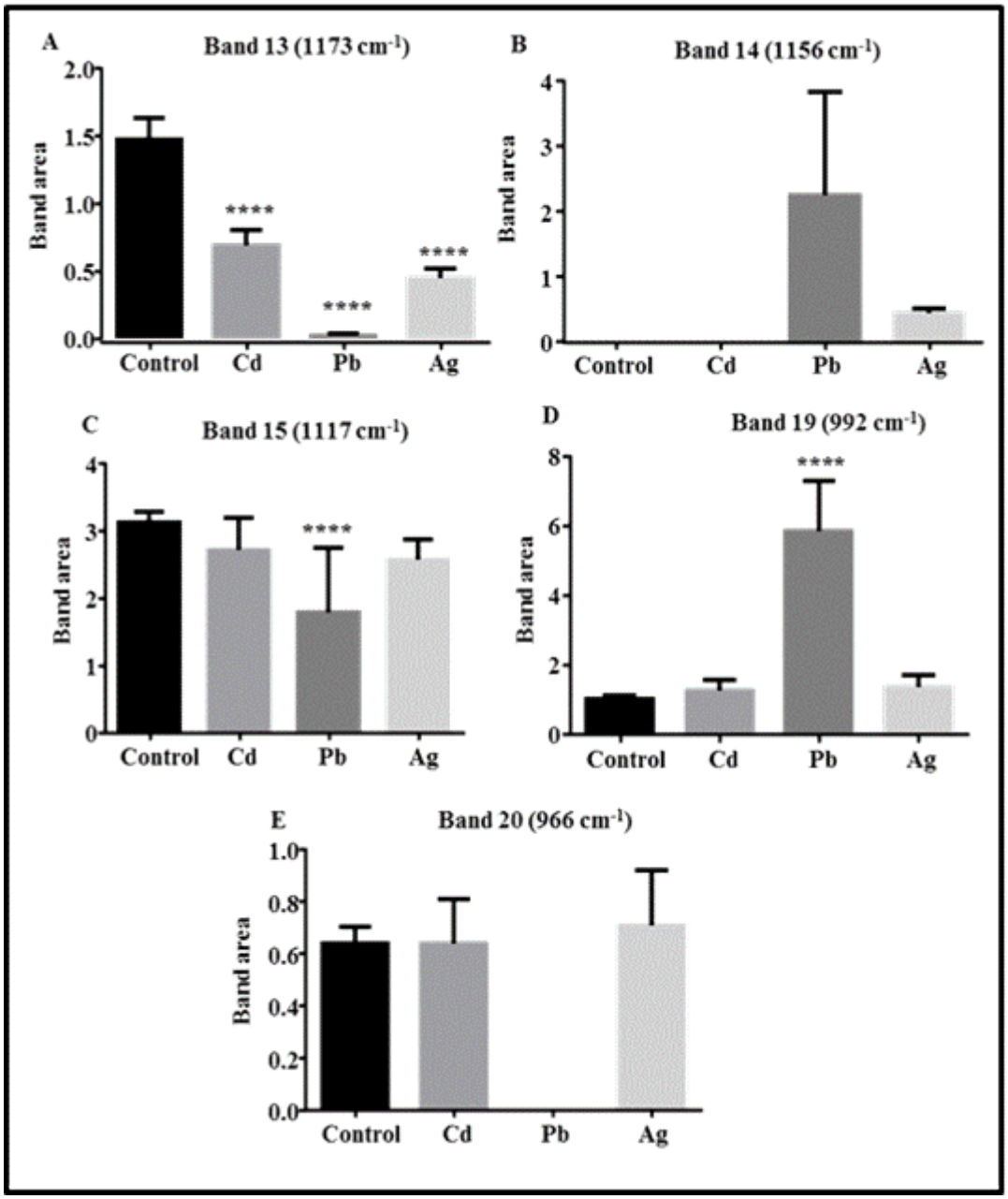

Figure 5. Bar diagram representing the concentration information obtained from the band areas of control and heavy metal treated environmental *Acinetobacter* sp. in the 1200-900 cm^{-1} region. **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001 represent the degree of significance, which is against control for each heavy metal treated groups.

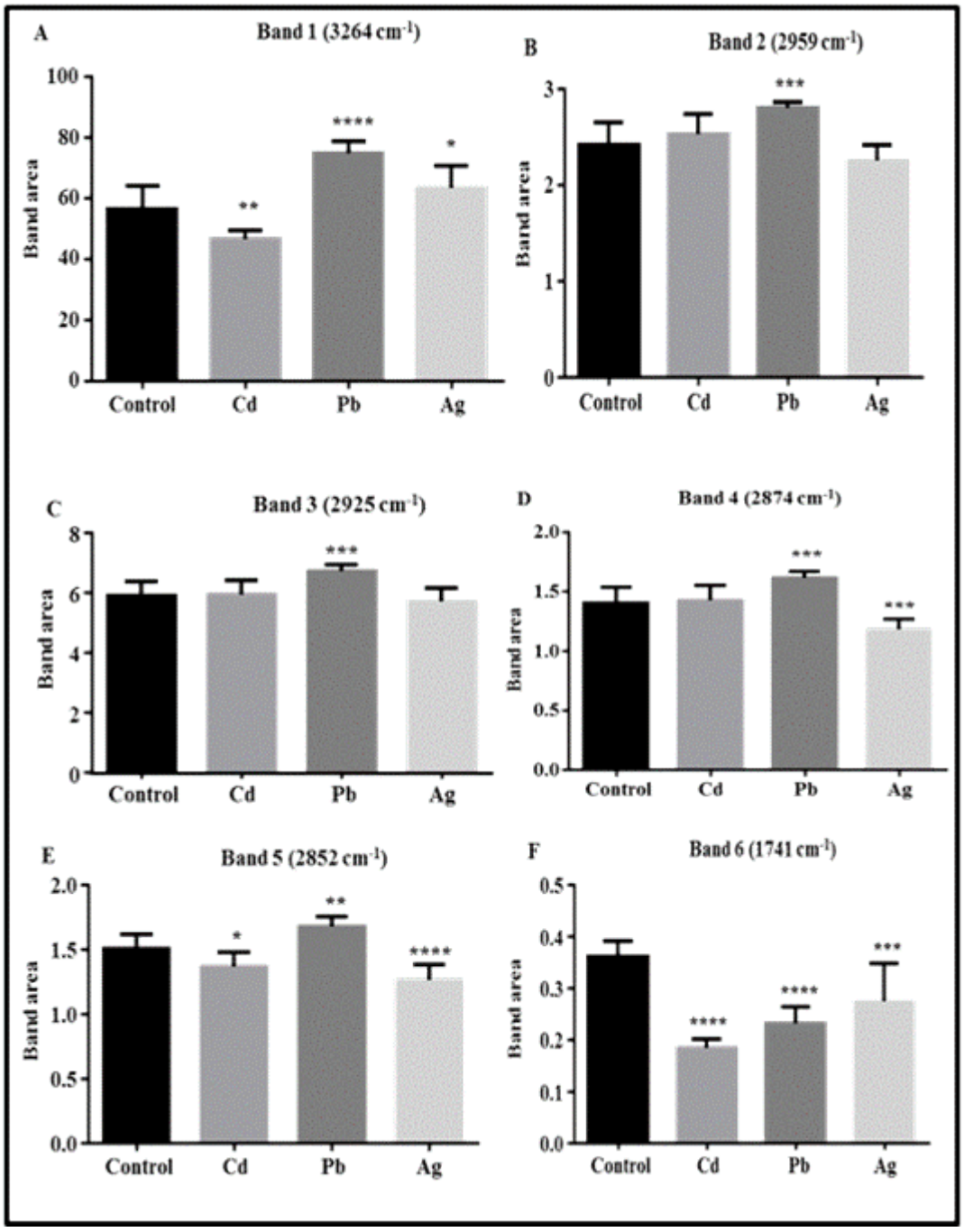

Figure 6. Bar diagram representing the concentration information obtained from the band areas of control and heavy metal treated *A. haemolyticus* ATCC 19002 in the 4000-1600 cm^{-1} region. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 represent the degree of significance, which is against control for each heavy metal treated groups.

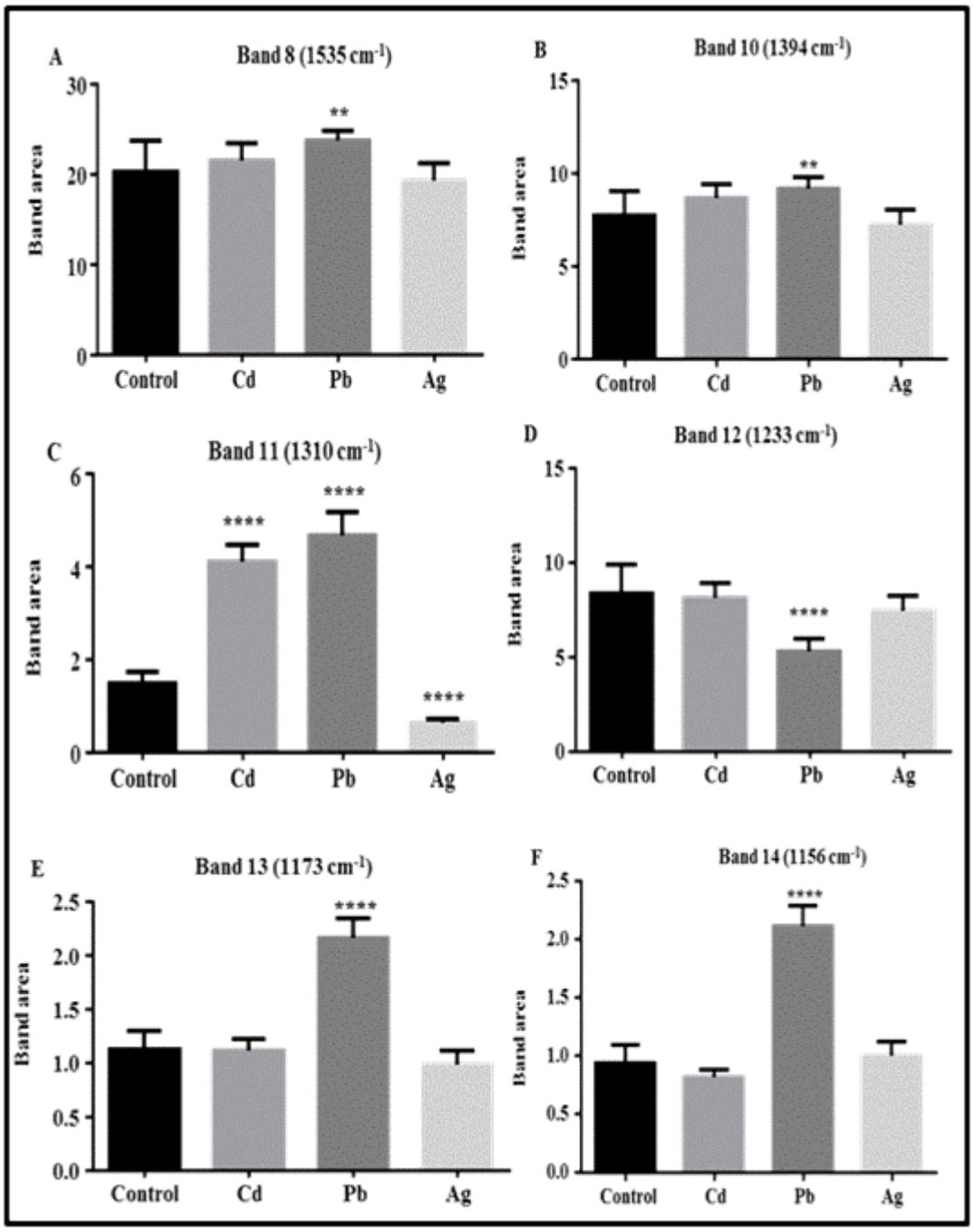

Figure 7. Bar diagram representing the concentration information obtained from the band areas of control and heavy metal treated *A. haemolyticus* ATCC 19002 in the 1600-1120 cm^{-1} region. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$ represent the degree of significance, which is against control for each heavy metal treated groups.

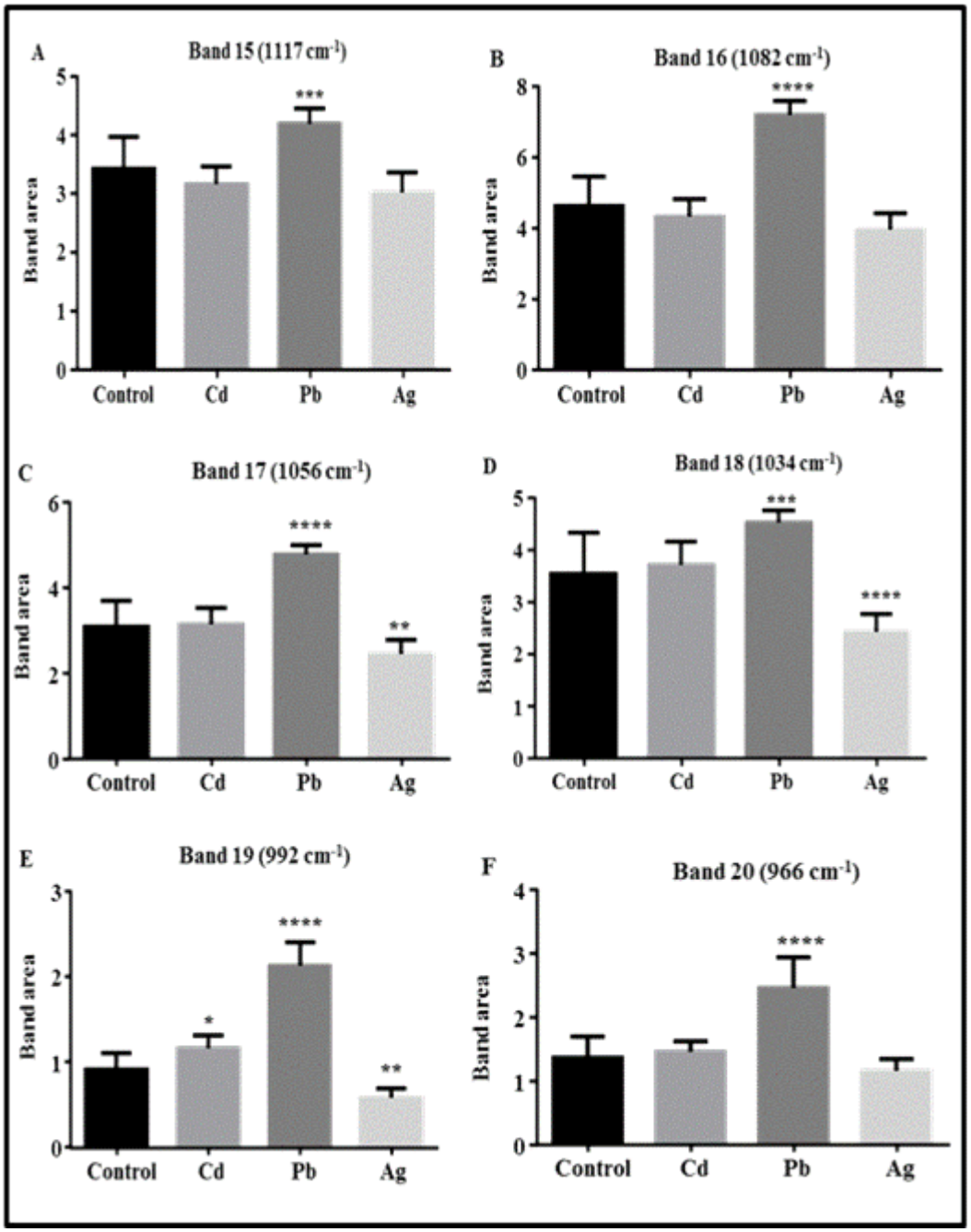

Figure 8. Bar diagram representing the concentration information obtained from the band areas of control and heavy metal treated *A. haemolyticus* ATCC 19002 in the 1120-900 cm^{-1} region. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 represent the degree of significance, which is against control for each heavy metal treated groups.

Table 5. The bandwidth values with significant differences between control and heavy metal treated environmental *Acinetobacter* sp. and *A. haemolyticus* ATCC 19002

Bandwidth values (cm^{-1}) for environmental *Acinetobacter* sp. (n=10)										
Band no	**Control vs. 7.8 µg/ml Cadmium**				**Control vs. 600 µg/ml Lead**			**Control vs. 15.63 µg/ml Silver**		
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**	**Pb Mean ± SD**	**% change**	***p*-value**	**Ag Mean ± SD**	**% change**	***p*-value**
5	5.71 ± 0.33	5.07 ± 0.57	12.62	*	5.05 ± 0.36	13.07	*	5.09 ± 0.69	12.18	ns
7	38.59 ± 0.65	40.31 ± 0.91	-4.27	**	44.71 ± 1.78	-13.69	****	39.71 ± 0.71	-2.82	ns
8	37.51 ± 0.36	37.64 ± 0.86	-0.35	ns	38.85 ± 0.68	-3.45	***	36.63 ± 1.06	2.40	ns
Bandwidth values (cm^{-1}) for *A. haemolyticus* ATCC 19002 (n=10)										
Band no	**Control vs. 80 µg/ml Cadmium**				**Control vs. 900 µg/ml Lead**			**Control vs. 15.63 µg/ml Silver**		
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**	**Pb Mean ± SD**	**% change**	***p*-value**	**Ag Mean ± SD**	**% change**	***p*-value**
3	18.63 ± 1.92	18.99 ± 0.58	-1.90	ns	20.82 ± 0.65	-10.52	*	18.72 ± 2.38	-0.48	ns

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, non-specific
The "-" indicates increases and the "+" shows decreases when compared to control group values

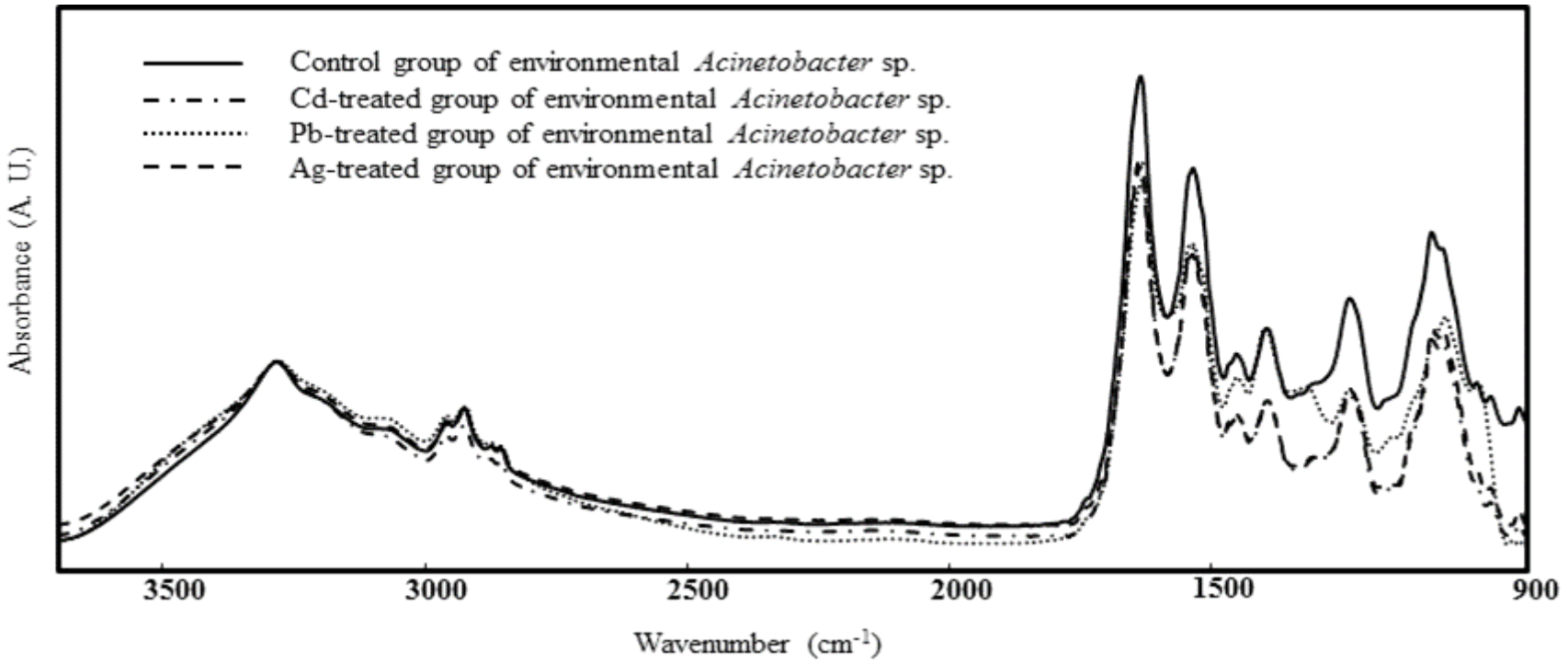

Figure 9. The average spectra of the control and heavy metal treated environmental *Acinetobacter* sp. in the 4000-900 cm^{-1} region. The spectra were normalized with respect to the amide A located at 3264 cm^{-1}.

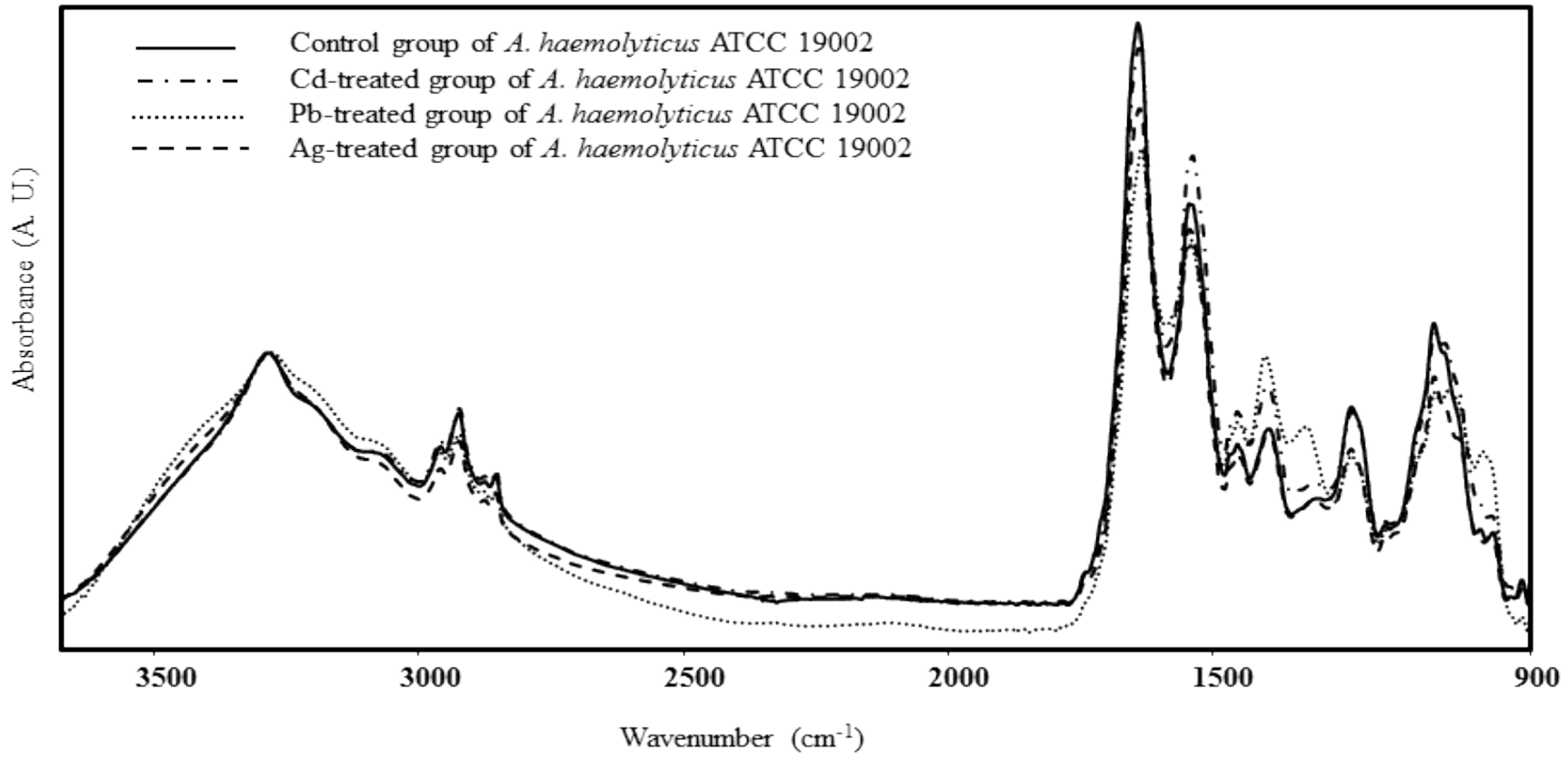

Figure 10. The average spectra of the control and heavy metal treated *A. haemolyticus* ATCC 19002 in the 4000-900 cm^{-1} region. The spectra were normalized with respect to the amide A located at 3264 cm^{-1}.

3.2.1.1 Changes in Cellular Proteins

The amide bands (at around 3264, 1639, 1535, and 1310 cm^{-1}) generated by vibrations of the functional groups in peptides give valuable information about secondary structure of polypeptides and proteins (Haris and Severcan, 1999; Barth, 2007). Additionally CH_3 symmetric stretching band (at 2874 cm^{-1}) mainly originating from cellular proteins (Cakmak et al, 2006; Ozek et al., 2014) were evaluated to obtain information on metal effects.

3.2.1.1.1 Aspect of Molecular Structure and Interactions

Protein structural changes are the most frequently encountered results in heavy metal toxicity (Poole and Gadd, 1989). Nonessential metals show great affinity to bind to thiol-containing groups of proteins (Deratani and Sebille, 1981; Hughes and Poole, 1989; Poole and Gadd, 1989). Especially bacterial membranes mostly contain sulfur-rich proteins (Morones et al., 2005).

In this study according to vibrational spectra it was found that there were significant structural changes in functional groups which were mainly related with proteins in the all heavy metal treated groups (Table 3 and 4). Proteins of two *Acinetobacter* strain differed under the influence of the same heavy metal. For instance, the significant (p <0.05) shift in the position of amide II band was only seen in Ag-treated *A. haemolyticus,* there was a similar shift in the spectrum of another bacteria exposed to Cd (Huang et al., 2013). The amide II band is the combination of primarily N–H bending with a contribution from C–N stretching vibrations (Haris and Severcan, 1999; Barth and Zscherp, 2002). The amide II band is mostly affected by amino acid side chain vibrations like amide I band. Nevertheless, amide II vibrations are not as sensitive as amide I vibrations in terms of correlation between band position and secondary structure of proteins (Barth and Zscherp, 2002). Instead, it can give valuable information about general state of proteins (Carpenter and Crowe, 1989). The fine changes in protein structure arising from hydrogen bonding

will be primarily marked by the NH bending vibrations (Haris and Severcan, 1999; Barth and Zscherp, 2002) as it was measured in Ag-treated *A. haemolyticus* in this study.

Another important example, frequency downshift at the amide I band was only seen in Pb treated *A. haemolyticus* in this study. The similar alteration was also reported for Cr contacting *A. haemolyticus* EF369508 (Yahya et al., 2012), and shifts at the same band to higher values in other Cd and Pb treated bacteria were observed (Choudhary and Sar, 2009; Huang and Liu, 2013). The vibrations in amide I region (1600-1700 cm^{-1}) corresponding to polypeptide backbone of secondary-structure of a protein (Surewicz et al., 1993) are composed of many overlapping structures such as α-helices, β-sheets, turns and non-ordered or irregular structures (Haris and Severcan, 1999). If amide I absorption occurs in the spectral range between 1620-1640 cm^{-1}, proteins are said to be in β-sheet structure (Haris and Chapman, 1992; Surewicz et al., 1993; Haris et al., 1986; Susi and Byler, 1986; Tamm and Tatulian, 1997; Naumann, 2001). In the studied two *Acinetobacter* strain, amide I bands of control groups observed at around 1639 cm^{-1}. Likewise, in a previous report, the bands below 1640 cm^{-1} may also originate from vibrational motions of α -helical structures (Torii and Tasumi, 1992). Thus we cannot say that this downshift in Pb-treated *A. haemolyticus* was a direct result of changes in β-sheet structure of bacterial proteins. In our case -downshift of nearly 3 cm^{-1}- resembles more with the cases mentioned by Barth and Zscherp (2002): downshift of 1 cm^{-1} for C=O groups were related with a binding of several aliphatic compounds. Furthermore, amino sugars (with N-acetyl/glucuronamide groups) from cell associated polysaccharides could also manifest this band (Beech et al., 1999; Kazy et al., 2009).

In other studies especially dealing with metal contaminated environments, Cd-treated (Choudhary and Sar, 2009; Huang et al., 2013; Huang and Liu, 2013) and Pb-treated bacteria (Huang and Liu, 2013) showed frequency shifts in amide A band, on the contrary, in our study Pb and Ag-treated *Acinetobacter* strains had shift at this band but not Cd-treated one. Since the amide A band is a broad and strong band (~3300

cm^{-1}) due to the stretching of the N–H bond of amino groups along with O–H vibrations of the hydroxyl groups from proteins and polysaccharides (Pagnanelli et al., 2000), the presence of these shifts was ascribed to the involvement of the bounded amino and hydroxyl groups during metal binding to bacterial surface (Kazy et al., 2006; Huang and Liu, 2013). It was also supported by the knowledge about that specifically the frequency of this band depends on the strength of the hydrogen bond rather than the conformation of the polypeptide backbone (Barth and Zscherp, 2002; Barth, 2007): hydrogen bonding -especially to PO_2^- groups- lowers the frequency of stretching vibrations by 3-20 cm^{-1} (Colthup et al., 1975; Brown and Peticolas, 1975; Arrondo et al. 1984; Pohle et al. 1990; George et al. 1994) as in the case of Pb- treated environmental *Acinetobacter* sp. (nearly 5 cm^{-1} downshift), but increases that of bending vibrations (Colthup et al., 1975) like in Ag-treated *A. haemolyticus* (nearly 8 cm^{-1}) in this study.

3.2.1.1.2 Aspect of Concentration of Functional Groups

Under normal conditions intracellular metal concentrations are regulated by nonspecific membrane transport mechanisms (Nies and Silver, 1995). When metal concentration were reached to toxic levels in the cell, however, synthesis of specific ion efflux systems start to exclude (Bruins et al., 2000). In addition, proteins in nuclear region were increased most probably to protect the DNA from heavy metal ions (Feng et al., 2000).

In this study changes in concentrations of functional groups which were related with bacterial proteins in all heavy metal treated groups were also observed in different levels: In Pb-treated environmental *Acinetobacter* sp. only reducing concentration at amide I band (Fig. 4C) in this study, on the other hand, in another study with heavy metal treated environmental microflora (Nithya et al., 2011), reduction in amide I was reported for Cd-exposed bacterial cells. This means that there was considerable decrease in the interaction between the proteins and peptides (Mecozzi et al., 2007)

in the case of Pb-treated environmental *Acinetobacter* cells; we did not detect significant difference in the other metal-treated cells.

The amide A bands were significantly increased in both Ag-treated *Acinetobacter* strain and also in Pb-treated *A. haemolyticus*, while in Cd-treated *A. haemolyticus* it was significantly decreased when compared to untreated control groups (Fig. 4A and 6A). Increased or reduced concentrations of functional groups related with amide A band was also reported for Pb and Cd treated sediment bacteria by Nithya et al. (2011) like in the case of Cd-, Pb-, and Ag-treated *Acinetobacter* cells. It is known that the amide A band is also affected by hydration status of the sample. Since *Acinetobacter* cells were dried by N_2 gases before measuring by using ATR-FTIR spectroscopy, the contribution of water to this band can be ignored. Therefore all alterations were thought to be mainly related with proteins and polysaccharides.

It can be said that in environmental strain a few functional groups changed their concentration; on the other hand, almost all functional groups changed their concentrations in *A. haemolyticus* under the effect of the three heavy metals. Especially *A. haemolyticus* was more to be influenced by Pb as indicated via increased protein band area, while there was not noticeable change in Cd-treated environmental strain. Furthermore, the changes at the bands of amide A and amide III were common in all heavy metal treated groups of *A. haemolyticus*.

3.2.1.1.3 Aspect of Conformational Freedom and Flexibility

Bandwidth changes of amide I and II represent the conformational freedom of proteins (Wharton, 2000; Barth and Zscherp, 2002). Environmental *Acinetobacter* showed conformational flexibility of cellular proteins after heavy metal exposure (Table 5). However, there were not any significant changes in metal treated groups of *A. haemolyticus* ATCC: 19002. Thus for amide I, both Cd-treated and Pb-treated environmental groups had significantly broader bands. For amide II, only Pb-treated group was significantly broader than control group. The studies with metalloproteins

have reported that the amide II band greatly changes in the metal free forms than metal-bound forms (Jackson et al., 1991; Alvarez et al., 1987; Hadden et al., 1994). This changes most probably originated by flexibility/mobility in the proteins; they contain little or no alteration in secondary structure (Haris and Severcan, 1999). Besides, toxic metal ions inactivate the proteins interfering with important cellular functions by replacing essential metal ions (Nieboer and Fletcher, 1996). Conformational freedom did not change in Ag-treated ones. It is known that flexible structures will give broader bands than rigid structures as binding of molecules to proteins decreased the conformational freedom (reviewed by Barth, 2007). Environmental *Acinetobacter* strain when exposed to Cd and Pb, had increased protein flexibility.

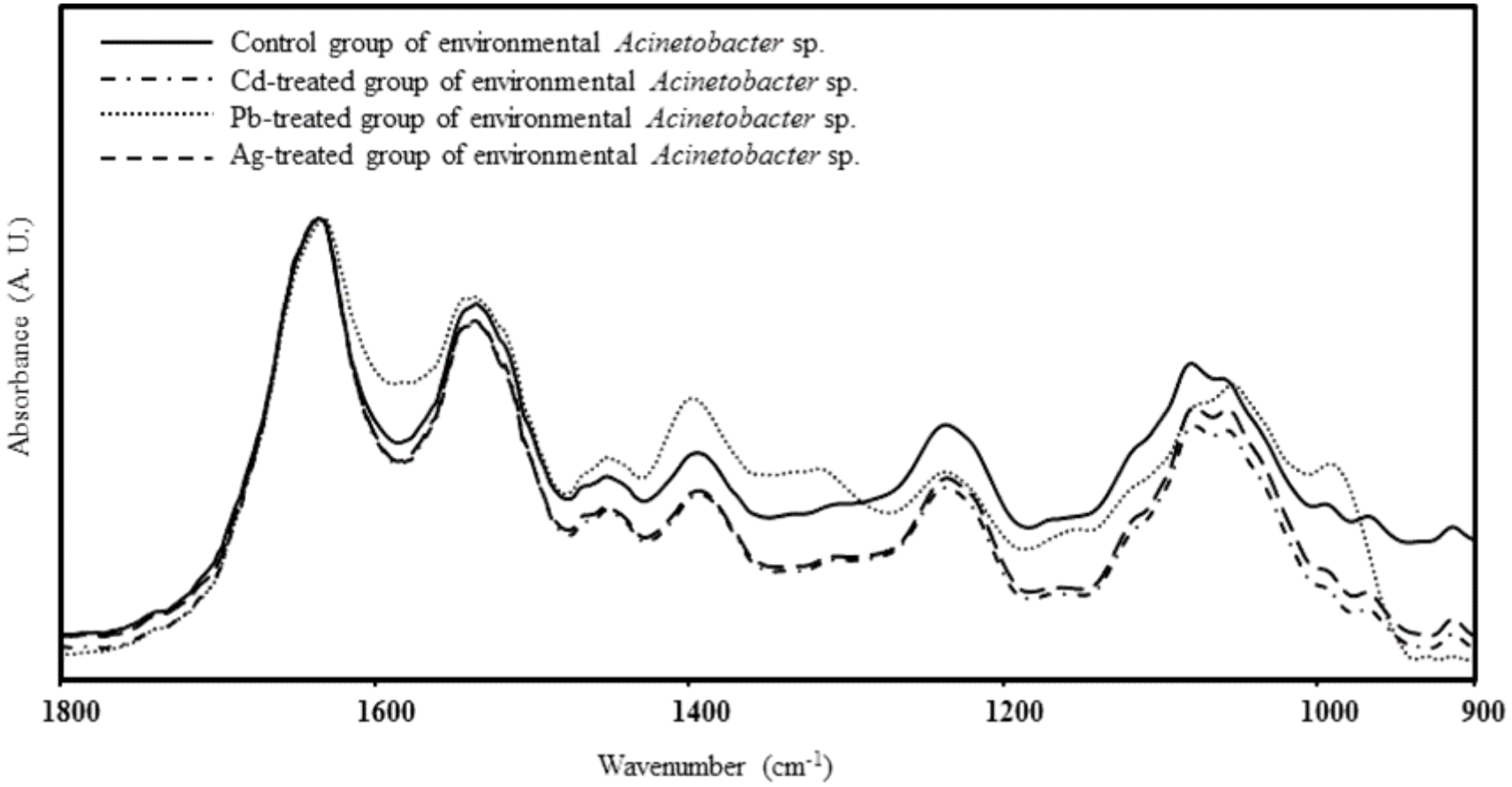

Figure 11. The average spectra of the control and heavy metal treated environmental *Acinetobacter* sp. in the 1800-900 cm^{-1} region. The spectra were normalized with respect to the amide I located at 1639 cm^{-1}.

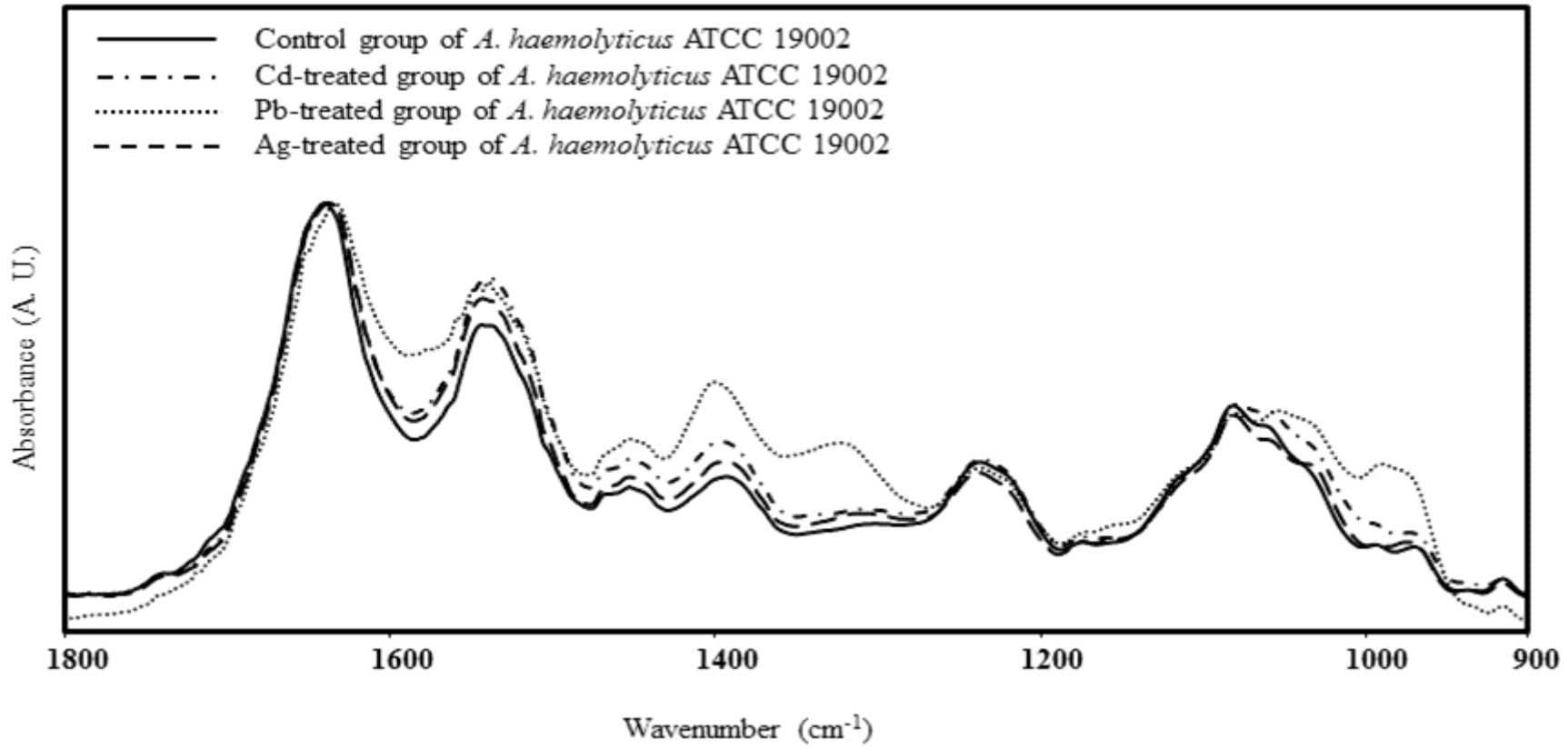

Figure 12. The average spectra of the control and heavy metal treated *A. haemolyticus* ATCC 19002 in the 1800-900 cm^{-1} region. The spectra were normalized with respect to the amide I located at 1639 cm^{-1}.

3.2.1.2 Changes in Cellular Lipids and Fatty Acid Components

Under the stress conditions, such as heavy metal toxicity, microorganisms can alter their lipid biochemistry especially to change membrane fluidity. This type of response includes changes of fatty acid composition and inhibition of lipid biosynthesis and lipid peroxidation (Denich et al., 2003; Heipieper et al., 2003; Markowicz et al; 2010; Guschina and Harwood, 2006).

The strong bands are functions of the antisymmetric and symmetric CH_2 stretching modes of the acyl chains (at around 2925 and 2852 cm^{-1}; respectively), and the minor bands are that of the antisymmetric stretching vibrations of the terminal CH_3 (at around 2959 cm^{-1}) groups of fatty acids. These bands give valuable information on cellular lipids and other fatty acid containing components (Boyar and Severcan, 1997; Severcan, 1997; Severcan et al., 2005). In addition, the ester group vibrations (C=O stretching; at around 1741 cm^{-1}) (Boyar and Severcan, 1997; Severcan et al., 2005; Korkmaz and Severcan, 2005) and the bending vibrations of CH_2 groups (at around 1451 cm^{-1}) (Jiang et al., 2004; Cakmak et al., 2006) are also used to evaluate cellular lipids in bacterial cells.

3.2.1.2.1 Aspect of Molecular Structure and Interactions

The band at 1451 cm^{-1} is characteristic for the CH_2 scissoring motion in lipids (Jiang et al., 2004). This group of vibrations arises mainly from cell envelope components (peptidoglycan, teichoic acid, LPS, phospholipids, and membranes) (Jiang et al., 2004; Yu and Irudayaraj, 2005; Kamnev et al., 1999). A pronounced shift to lower values in all heavy metal treated groups except Ag-treated *A. haemolyticus* occurred in this region (Table 3 and 4). This shift was similar with Cr-treated *A. junii* and Cd-treated *Pseudomonas* sp. and most probably due to the binding of metals to lipoproteins (Paul et al., 2012; Choudhary and Sar, 2009).

The absorption peaks at 2959, 2925, and 1741 cm^{-1} did not show significant changes in their positions in any group. In the other studies, though, there were shifts for CH_2 antisymmetric stretching (2925 cm^{-1}) in Cd-treated and Pb-treated bacteria (Huang and Liu, 2013), and for C=O stretching of triglycerides (1741 cm^{-1}) in Cd treated bacteria (Huang et al., 2013).

Since the frequency shift of the acyl chain methylene symmetric and antisymmetric stretching bands near 2852 and 2925 cm^{-1} give direct information about order/disorder transitions of membranes of bacteria (Schultz and Naumann, 1991; Casal and Mantsch, 1984), it can be said that "state of order" of the membranes (cytoplasmic membrane and outer membrane) were higher than control groups for Pb- and Ag treated environmental *Acinetobacter* strain with significantly decreasing frequency values at 2852 cm^{-1} (Table 3). This type of stabilization of membrane was most probably resulted due to the formation of ionic bonds between Ag and Pb cations and negative charges on the phospholipids. This nonspecific binding of the toxic metal ions to the membrane also prevented them entering to the cells.

Since no additional significant changes observed apart from these bands (at 1451 and 2852 cm^{-1}), there were not any remarkable conformational changes determined in the membranes of studied bacteria against resistance to Cd, Pb, and Ag. These may imply that the heavy metal resistance in part is a function of nonspecific binding. For all tested groups of *Acinetobacter*, the other modifications of fatty acid structure could be part of a defense or/and repair mechanism aimed at reducing the damage caused by heavy metal stress.

3.2.1.2.2 Aspect of Concentration of Functional Groups

The changes in the concentration of functional groups in lipid related components in the heavy metal exposed cells differed with respect to each metal and differed between the two bacteria. Shared situation in all metal-treated *Acinetobacter* groups was the reduction of the concentrations related to C=O stretching of ester groups in

triglycerides mainly due to cell walls and cellular membranes at around 1741 cm^{-1} (Naumann, 1984). Decreasing fatty acid content inferred to be most probably due to lipid peroxidation as a result of metal exposure was reported for Cd as in the study of Markowicz et al. (2010).

In turn, the band of CH_2 symmetric stretching (at 2852 cm^{-1}) observed in different levels (increase or decrease) after each metal exposure in *A. haemolyticus* (Fig. 6E) but not in environmental *Acinetobacter* isolate.

Besides, only Pb-treated *A. haemolyticus* had significantly increased area at the bands for CH_3 antisymmetric stretching (2959 cm^{-1}; Fig. 6B) and CH_2 antisymmetric stretching (2925 cm^{-1}; Fig. 6C) modes of lipids; and only Ag-treated environmental *Acinetobacter* had significantly increased area at the bands for CH_2 bending (1451 cm^{-1}; Fig. 4D).

In brief, Pb-treated *A. haemolyticus* cells prominently were defined by rising fatty acid concentration. On the contrary, there were reduced fatty acid content detected in Cd and Ag treated bacteria as well as Pb-treated environmental *Acinetobacter* isolate. This increased content for fatty acids was also reported for Pb-treated sediment bacteria but not for Cd treated cells in the study of Nithya et al. (2011).

3.2.1.2.3 Aspect of Conformational Freedom and Flexibility

Bacteria can modify their acyl chain structure by changing the level of saturation, type of saturation (cis to trans), the level of branched structure, type of branching and the length of acyl chain (Denich et al., 2003; Grogan and Cronan, 1997; Heipieper et al., 2003; Kim et al., 2001).The bandwidth value of the CH_2 stretching bands (at 2925 and 2852 cm^{-1}) represents the changes of translational and rotational mobility of the fatty acid chains (Schultz and Naumann, 1991; Boyar and Severcan, 1997; Severcan et al., 2005; Korkmaz and Severcan, 2005). In this context, only in Cd-treated and Pb-treated groups of environmental *Acinetobacter* cells had significantly

narrower bandwidth for CH_2 symmetric stretching (at 2852 cm^{-1}) than in control group (Table 5). This means that Cd and Pb exposure cause a reduction in the lipid fluidity in the environmental strain of *Acinetobacter* (Cakmak et al., 2006; Severcan et al., 2005). On the other hand, significantly broadened band (at 2925 cm^{-1}) in Pb-treated *A. haemolyticus* indicated that Pb treatment cause an increase in the lipid fluidity for the clinical strain of *Acinetobacter* (Table 5) (Severcan et al., 2005; Korkmaz and Severcan, 2005).

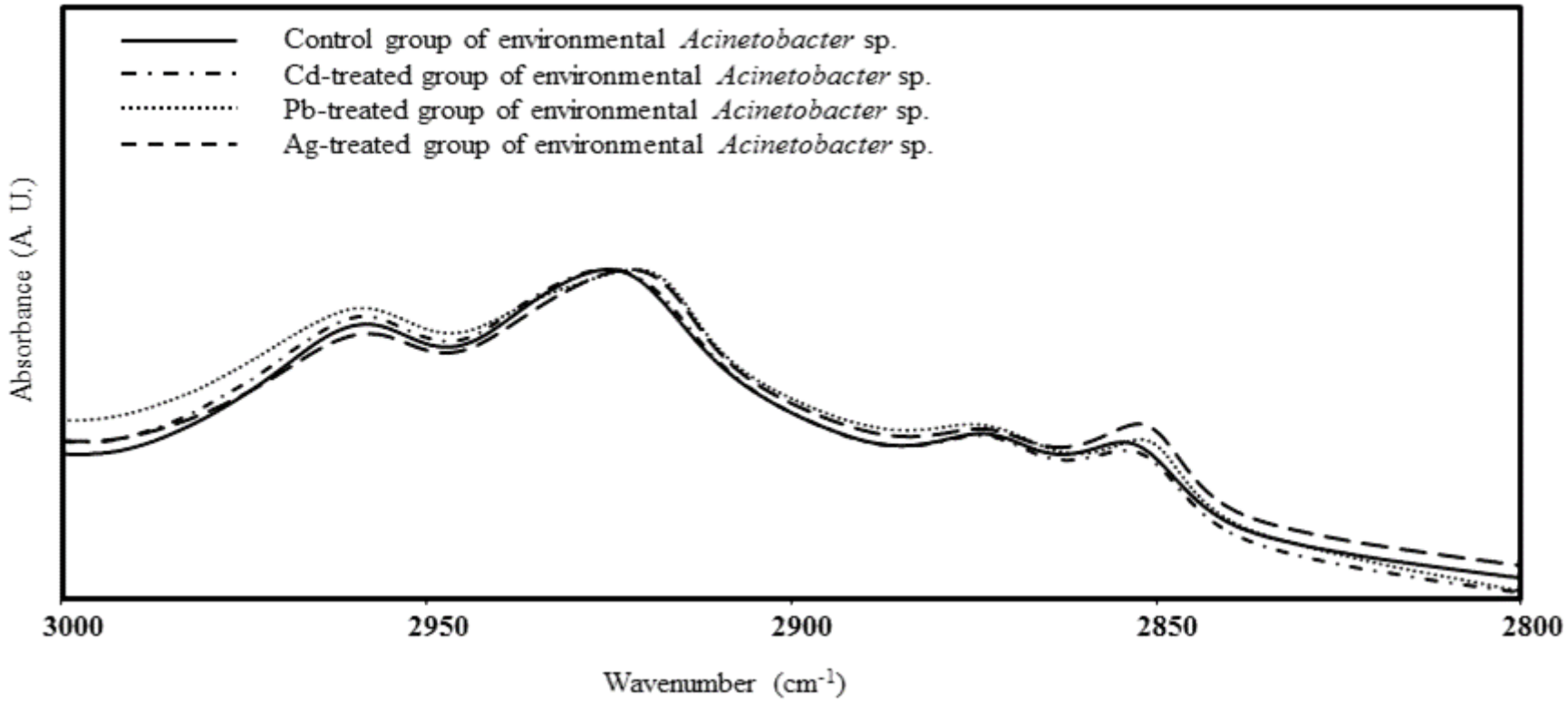

Figure 13. The average spectra of the control and heavy metal treated environmental *Acinetobacter* sp. in the 3000-2800 cm^{-1} region. The spectra were normalized with respect to the CH_2 asymmetric stretching band located at 2925 cm^{-1}.

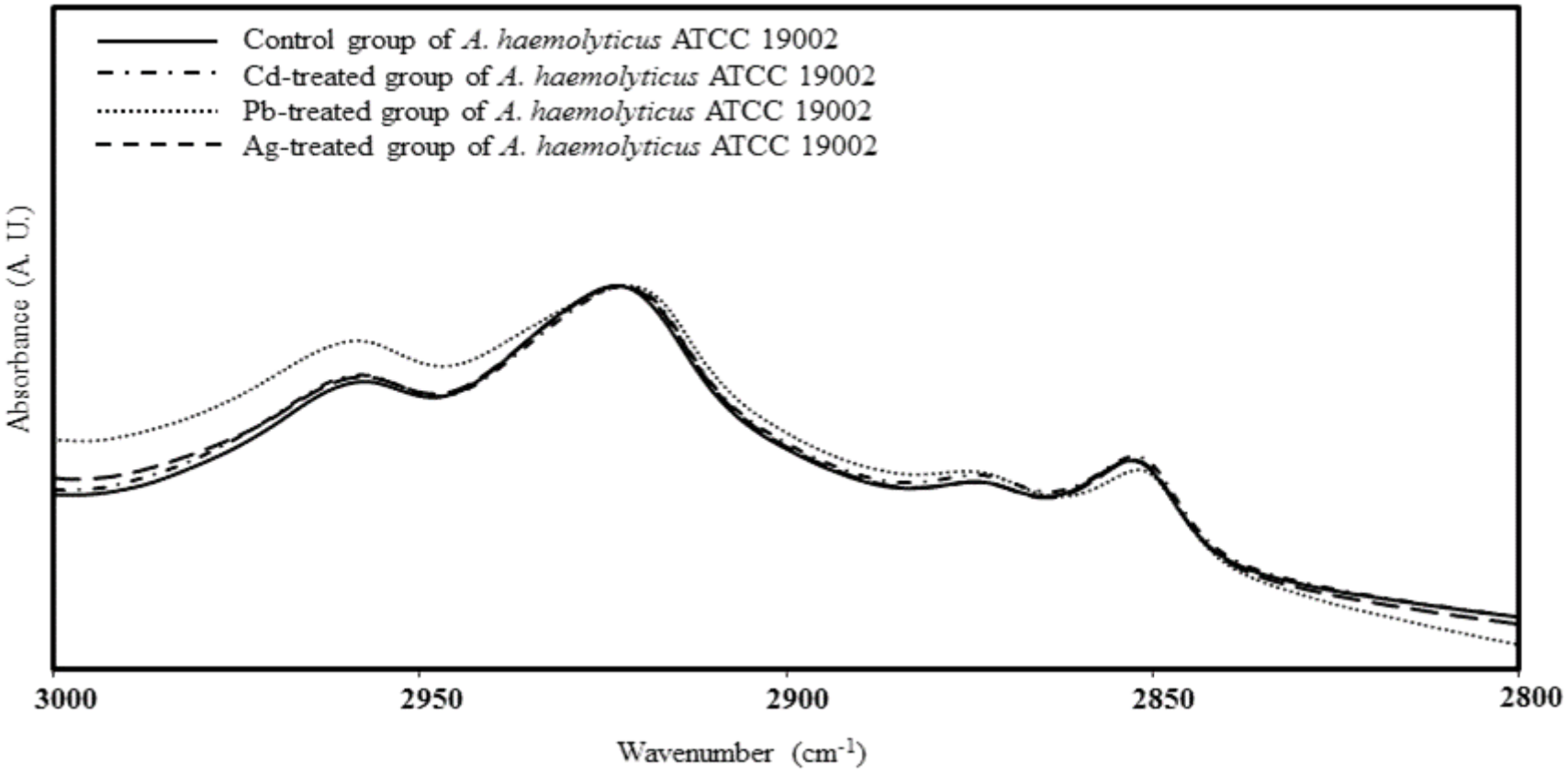

Figure 14. The average spectra of the control and heavy metal treated *A. haemolyticus* ATCC 19002 in the 3000-2800 cm^{-1} region. The spectra were normalized with respect to the CH_2 asymmetric stretching band located at 2925 cm^{-1}.

3.2.1.3 Changes in Genetic Elements

Significantly altered absorption peaks at 1233, 1174, 1117, 1082, 994, and 966 cm^{-1} revealed changes in nucleic acids of two *Acinetobacter* strains after exposure to sub-lethal Cd, Pb, Ag concentrations. The bands at 1250–1000 cm^{-1} and region containing vibrations of the sugar–phosphate chains are sensitive to nucleic acid backbone conformation (*A*-, *B*- or *Z*-form) (Banyay et al., 2003). In addition, the bands in the 1000–800 cm^{-1} region, which contains the sugar–phosphate backbone related vibrations, are used as marker for nucleic acid sugar puckering modes (*N*- and *S*-type) (Banyay et al., 2003).

3.2.1.3.1 Aspect of Molecular Structure and Interactions

Heavy metal toxicity partly results from alterations in the structural conformation of nucleic acids (Poole and Gadd, 1989). The antisymmetric and symmetric stretching vibrations of PO_2 (at 1233 and 1082 cm^{-1}, respectively) mostly originate from phosphodiester backbone of cellular nucleic acids; the contribution of phosphate residues in membrane lipids can be disregarded (Rigas et al. 1990; Wong et al., 1991; Wang et al., 1997). The strong peak at around 1233 cm^{-1} was significantly shifted after treatment with sub-inhibitory concentration of Ag in both *Acinetobacter* but the shifts were in opposite energy values (Table 3 and 4). There were not any changes in the Cd- or Pb-treated *Acinetobacter* groups in terms of PO_2 antisymmetric stretching. On the other hand, in a study with environmental *Pseudomonas* sp., a shift in Cd treated group was reported previously (Choudhary and Sar, 2009). This band is sensitive to nucleic acid backbone conformation and irrelevant from nucleobase and sugar vibrations (Banyay et al., 2003). Furthermore it allows the determination of structural transitions among DNA forms (Naumann et al. 1996). In this study, the absorption peak for this band was nearly at 1240 cm^{-1} which indicates *A*-form double helix (Taillandier and Liquier, 1992).

The band at around 1082 cm^{-1} significantly shifted to lower energy in all heavy metal-treated environmental *Acinetobacter* groups (Table 3) and Pb-treated *A. haemolyticus* (Table 4). Similar frequency shift was also observed in other studies with heavy metal treated environmental bacteria (Nithya et al., 2011) and in the Cr-treated *A. haemolyticus* (Yahya et al., 2012). This band is less sensitive to conformational changes in nucleic acids than antisymmetric PO_2 stretching (Banyay et al., 2003). The appeared downshift of band position measured in this suggested contribution of phosphate groups to hydrogen bonding (Barth and Zscherp, 2002; Severcan et al., 2005; Korkmaz and Severcan, 2005). This strengthening of hydrogen bonding indicated structural changes in DNA/RNA conformation (Awayda et al., 2004).

The small band at around 1173 cm^{-1} is mainly assigned to sugar–phosphate backbone vibrations with a contribution more on the sugar moiety side (Pohle and Fritzsche, 1980). There was no significant difference in frequency value in environmental *Acinetobacter* sp. On the other hand only Cd-treated *A. haemolyticus* had significant downshift in band position which appeared to be due to the structural variation in the nucleic acids (Table 4) as pointed out in the study of Nithya et al. (2011). This band specifically used for identification of *A*-form of nucleic acids (Banyay et al., 2003).

There was significant frequency shift of the vibrational modes of ribose at nearly 1117 cm^{-1} in only Pb-treated group to higher energy (at around 1120 cm^{-1}) with respect to control in environmental *Acinetobacter* (Table 3). Double stranded polyribonucleotides absorption was reported at around 1119 cm^{-1} as in the case of Pb-treated environmental *Acinetobacter* (Tsuboi, 1969). On the other hand Pb- and Ag-treated *A. haemolyticus* had lower energy than control group (Table 4).

The band at around 992 cm^{-1} represents ribose-phosphate main chain vibrations (Banyay et al., 2003). There were remarkable significant (Table 3 and 4) decreases in the frequency values in only Pb-treated cells for both *Acinetobacter* groups with respect to controls. Actually the absorption peaks at 992 and 966 cm^{-1} detected in control bacteria spectrum disappeared in the spectrum of Pb-treated environmental

strain. Instead, a new band at 979 cm^{-1} was located in Pb-treated environmental strain. According to previous studies *A*-form of DNA is responsible for absorption peaks at around 977 and 968 cm^{-1} (reviewed by Banyay et al., 2003). We assumed that the two bands (at 992 and 966 cm^{-1}) which were related with nucleic acids congregated and seen as one band (at 979 cm^{-1}) after sub-lethal Pb exposure in environmental *Acinetobacter*. The relatively small downshift in Pb-treated *A. haemolyticus* when compared to environmental strain most probably arisen from vibrations of –OH group in ribose-phoshate chain (Liquier et al., 1991). Furthermore, the band at 966 cm^{-1} is marked for main chain vibrations of nucleic acids (Tsuboi, 1969; Ci et al., 1999; Banyay et al., 2003; Cakmak et al., 2006). This band showed significant shift to a higher value only in Pb-treated *A. haemolyticus*.

In summary, it was shown that Pb caused a highly significant change in nucleic acid vibrations among other tested metals.

3.2.1.3.2 Aspect of Concentration of Functional Groups

In terms of changes in concentrations for nucleic acid related functional groups, all heavy metal treated groups exhibited different effects: Band area values at 1173 cm^{-1} of all metal-treated environmental *Acinetobacter* were significantly less than control group (Fig. 5A). On the other hand, only Pb treated *A. haemolyticus* ATCC: 19002 had highly significant increase compared to control group (Fig. 7E). This was similar to the study of Pb treated bacteria (Nithya et al., 2011). Pb treated *A. haemolyticus* showed increase in sugar concentration attributed to nucleic acids, reverse was recoreded in environmental *Acinetobacter* sp for the three metals. Another similarity between Cd- and Pb-treated environmental *Acinetobacter* was in the reduced concentration of the PO_2 antisymmetric stretching (at 1233 cm^{-1}; Fig. 4F).

Reduced band area at 1233 and 1173 cm^{-1} and increased at that of 992 cm^{-1} were also common between both Pb-treated *Acinetobacter* strains, as compared control. Only Pb treated group showed significant change in the band area (at 1117 cm^{-1}) analysis

for both of the bacteria groups (Fig. 5C and 8A). In turn *A. haemolyticus*, the changes (increases or decreases) for the band at 992 cm^{-1} was detected in all heavy metal treated groups. We were able to see the band at 966 cm^{-1} was significantly (p <0.0001) sharpened in Pb-treated *A. haemolyticus* similar to as described in a previous study reporting Cd-treated environmental *Pseudomonas* sp. (Choudhary and Sar, 2009). However, total disappearance of this band in our Pb-treated environmental *Acinetobacter* strain was curious. In short, in terms of nucleic acid contents higher number of changes was observed in Pb-treated bacterial cells. Especially when we evaluated the backbone marker bands (1233, 1173, 1117, and 1082 cm^{-1}), decreased nucleic acid content were prominent in all metal-treated environmental *Acinetobacter*, while there was an increased concentration of nucleic acids in only Pb-treated *A. haemolyticus*.

3.2.1.4 Changes in Cell Wall and Other Surface Layers

Cell wall is the first cellular structure to contact with metal ions (Chakravarty and Banerjee, 2012). The complexation between various functional groups present on the cell surface and metal ions could be identified by FTIR analysis (Huang and Liu, 2013) by evaluating specified bands at 1394, 1156, 1056, 1034 cm^{-1}.

3.2.1.4.1 Aspect of Molecular Structure and Interactions

Toxic metals are excluded from cells by alterations of permeability barriers that include cell envelope and other surface layers (such as capsule and biofilm) of bacteria (Rouch et al., 1995; Ji and Silver, 1995). Bacteria can keep toxic metal ions away from sensitive cellular components with the help of these protective layers (Bruins et al., 2000). Proteins located in periplasmic space and outer membrane cause resistance by periplasmic binding or extracellular sequestration of heavy metal ions (Silver and Walderhaug, 1992; Silver and Ji, 1994). Besides, biosorption which

is another resistance mechanism involves all cell wall components: peptidoglycan layer, outer and inner membranes (Wang et al., 2006).

The weak band observed at nearly 1156 cm^{-1} is assigned to sugar ring vibration (Gao and Chorover, 2009) mainly from extracellular polysaccharides (EPS) (Nichols et. al., 1985; Quiles et al., 2010). There were not any related bands in control and Cd-treated groups of environmental *Acinetobacter* sp., although, it appeared in Pb-treated and Ag-treated (at around 1155 and 1161 cm^{-1}; respectively) environmental *Acinetobacter* sp. (Table 3). For *A. haemolyticus*, it was present in all heavy metal-treated groups and control group, however, the only significant change was observed in the case of Pb-treatment as a position downshift (Table 4). Nonspecific binding of metals to the outer membrane and EPS coatings also protects bacteria naturally (Beveridge and Murray, 1976; Hoyle and Beveridge, 1983; Scott et al., 1988; Scott and Palmer, 1988; Scott and Palmer, 1990). Especially in gram-negative bacteria, EPS have an important role for metal biosorption and sequestration (Silvestry-Rodriguez et al., 2008; Pandey et al., 2011) and *Acinetobacter* species are capable of biofilm formation (Pandey et al., 2011). It is known that metal exposure can induce the production of EPS (Kidambi et al., 1995; Wuertz et al., 2001). Although this type of resistance specifically for Cd ions was experimentally proven for many bacteria (Scott et al., 1988; Scott and Palmer, 1988; Mergeay, 1991), EPS related band was not seen in Cd-treated environmental *Acinetobacter* in this study. By contrast, Pb- and Ag-treated groups had this band at different positions. Nevertheless Pb-treated group had the band nearer to original EPS band position than Ag-treated group. Furthermore, band area analysis also showed that Pb-treated bacteria had more EPS content than Ag- treated bacteria. In turn, only Pb-treated bacteria had slight shift with increasing band area in *A. haemolyticus*. Our results showed that Pb was the most EPS production inducing heavy metal compared to Cd and Ag.

It can be said that the shift due to symmetric stretching of COO^- vibration at 1394 cm^{-1} was common in all the heavy metal treated groups of environmental *Acinetobacter* sp., although they were remarkably different in heavy metal treated *A.*

haemolyticus. This band mainly originated from amino acid side chains from peptidoglycan and capsule (Melin et al., 2001; Helm and Naumann 1995).

C-O-C and P-O-C symmetric stretching of polysaccharides on capsule and peptidoglycan were indicated with the band at 1056 cm^{-1}. There was a significant shift to the lower energy value only in Pb-treated group for environmental *Acinetobacter* sp. (Table 3); on the other hand, all heavy metal-treated *A. haemolyticus* had significantly lower values than the control group (Table 4). There was no change at this band position in the case of Cd-treated environmental *Acinetobacter* sp. though it was reported for Cd-treated environmental *B. cereus* (Huang et al., 2013).

The shift in the band position at nearly 1034 cm^{-1} to lower energy value was the only significant change which was observed in all the heavy metal-treated groups for both *Acinetobacter* strains in cell wall region of the ATR-FTIR spectrum (Table 3 and 4). This band is assigned to CO and CC stretching of alcohols and carboxylic acids, and also COH bending motions of polysaccharides (Mathlouthi and Koenig, 1986; Huang and Liu, 2013). In the study of Huang and Liu (2013), however, the shift to higher values was reported for Cd- and Pb-treated *Pseudomonas* sp.

Since the spectral vibrations at 1394, 1056 and 1034 cm^{-1} are mostly related to polysaccharides from peptidoglycan, the structural changes directly correlated with band positions may point out the changes in peptidoglycan layer of bacteria. Furthermore, the bands 1394 and 1034 cm^{-1} commonly downshifted in all metal-treated environmental *Acinetobacter*, whereas the bands 1056 and 1034 cm^{-1} commonly downshifted in all *A. haemolyticus* tested metals.

The other quite interesting situation about the cell wall related vibrations, all significantly changed band positions in both *Acinetobacter* examined groups were shift to lower energy values except the band at 1394 cm^{-1} which were observed as to higher energy values in Cd-treated *A. haemolyticus.*

Since the binding affinity of metal ions especially to extracellular polysaccharides depend on some factors such as the cation size/charge ratio, the bacterial polysaccharide charge, and the physical state of the biofilm (van Hullebusch et al., 2003), the observed spectral changes in this study were the results of differences among tested heavy metals and the bacteria.

3.2.1.4.2 Aspect of Concentration of Functional Groups

According to the analysis of the changes in the concentration of functional groups for cell wall and other surface layer related components in the heavy metal exposed cells, it can be said that they were totally different from each other. Besides, while Cd-treated environmental strain had no changes, Pb and Ag caused the remarkable changes especially in environmental strain (Fig. 5B, 7B, 7F, 8C and 8D).

CHAPTER 4

CONCLUSION

- Cd was the most toxic metal for environmental *Acinetobacter* isolate, Ag, however, exhibited the highest toxicity for clinical *Acinetobacter*. Pb was the least toxic for both *Acinetobacter* isolates. Also both *Acinetobacter* isolates had the same MIC value for Ag.

- Apparently, *Acinetobacter* cells protect themselves from mid-range level Cd toxicity by sequestration of metals by proteins and carbohydrates. This intracellular and extracellular sequestration was further supported by altered membrane permeability.

- The most pronounced environmental *Acinetobacter* cells after Ag exposure was EPS production. Thus it can be deduced that protection of environmental *Acinetobacter* sp. is mediated through arrest and immobilization of Ag in EPS.

- The prominent reductions in concentrations of proteins, lipids, carbohydrates and RNA recorded under the influence of Ag treatment were interpreted as cell size reduction indicators.

- Environmental *Acinetobacter* strain exposed to high concentration of Pb majority of cellular components had changed. The changes were more limited upon Cd and Ag. Interestingly Pb caused alteration in RNA structure.

- The dissimilarities in heavy metal-induced molecular changes for the two *Acinetobacter* strains (environmental and clinic) can be attributed to their different adaptation strategies which they assume in different environments.

REFERENCES

Abdel-El-Haleem, D. (2003). Acinetobacter: environmental and biotechnological applications. African Journal of Biotechnology, 2, 71–74.

Ahmedna, M., Marshall, W. E., Husseiny, A. A., Rao, R. M., Goktepe I. (2004). The use of nutshell carbons in drinking water filters for removal of trace metals. Water Research, 38 (4), 1062-1068.

Alomar, K., Landreau, A., Kempf, M., Khan, M. A., Allain, M., Bouet, G. (2010). Synthesis, crystal structure, characterization of zinc(II), cadmium(II) complexes with 3-thiophene aldehyde thiosemicarbazone (3TTSCH). Biological activities of 3TTSCH and its complexes. Journal of Inorganic Biochemistry, 104, 397–404.

Alvarez, J., Haris, P.I., Lee, D.C., Chapman, D. (1987). Conformational changes in concanavalin A associated with demetallization and α-methylmannose binding studied by Fourier transform infrared spectroscopy. Biochemica and Biophysica Acta, 916, 5-12.

Alvarez-Ordonez, A., Mouwen, D. J. M., Lopez, M., Prieto, M. (2011). Fourier transform infrared spectroscopy as a tool to characterize molecular composition and stress response in foodborne pathogenic bacteria. Journal of Microbiological Methods, 84, 369–378.

Alvarez-Ordonez, A., Prieto, M. (2010). Changes in ultrastructure and Fourier transform infrared spectrum of *Salmonella enterica* serovar typhimurium cells after exposure to stress conditions. Applied Environmental Microbiology, 76, 7598-7607.

Arrondo, J. L. R., Goñi, F. M., Macarulla, J. M. (1984). Infrared spectroscopy of phosphatidylcholines in aqueous suspension. A study of the phosphate group vibrations. Biochimica and Biophysica Acta, 794, 165–168.

Arrondo, J. L., Muga, A., Castresana, J., Goñi, F. M. (1993). Quantitative studies of the structure of proteins in solution by Fourier-transform infrared spectroscopy. Progress in Biophysics and Molecular Biology, 59, 23-56.

Asmub, M., Mullenders L. H. F., Hartwig, A. (2000). Interference by toxic metal compounds with isolated zinc finger DNA repair proteins. Toxicology Letters, 112-113, 227-231.

Awayda, M. S., Shao, W., Guo, F., Zeidel, M., Hill, W. G. (2004). ENaC-membrane interactions: regulation of channel activity by membrane order. The Journal of General Physiology, 123, 709-727.

Banyay, M., Sarkar, M., Gräslund, A. (2003). A library of IR bands of nucleic acids in solution. Biophysical Chemistry, 104, 477–488.

Barth, A. (2007). Infrared spectroscopy of proteins. Biochemica and Biophysica Acta, 1767, 1073-1101.

Barth, A., Zscherp, C. (2002). What vibrations tell us about proteins? Quarterly Reviews of Biophysics, 35 (4), 369–430.

Beech, I., Hanjagsit, L., Kalaji, M., Neal, A. L., Zinkevich, V. (1999). Chemical, structural characterization of exopolymers produced by *Pseudomonas* sp. NCIMB 2021 in continuous culture. Microbiology, 145, 1491–1497.

Beveridge, T. J., Murray, G. E. (1976). Uptake and retention of metals by cell walls of *Bacillus subtilis*. Journal of Bacteriology, 127, 1502-1518.

Bifulco, J. M., Shirey, J. J., Bissonnette, G. K. (1989). Detection of *Acinetobacter* spp. in rural drinking water supplies. Applied Environmental Microbiology, 55, 2214–2219.

Bouhedja, W., Sockalingum, G. D., Pina, P., Allouch, P., Bloy, C., Labia, R., Millot, J. M., Manfait, M. (1997). ATR-FTIR spectroscopie investigation of *E. coli* transconjugants β-lactams-resistance phenotype. FEBS Letters, 412, 39-42.

Boyar, H., Severcan, F. (1997). Oestrogen-phospholipid membrane interactions: FTIR studies. Journal of Molecular Structure, 408/409, 269-272.

Bragg, P. D., Rainnie, D. J. (1974). The effect of silver ions on respiratory-chain of *Escherichia coli*. Canadian Journal of Microbiology, 20, 883-889.

Braydich-Stolle, L., Hussain, S., Schlager, J.J., Hofmann, M. C. (2005). In vitro cytotoxicity of nanoparticles in mammalian germline stem cells. Toxicological Sciences, 88, 412-419.

Brown, E. B., Peticolas, W. L. (1975). Conformational geometry and vibrational frequencies of nucleic acid chains. Biopolymers, 14, 1259–1271.

Bruins, M. R., Kapil, S., Oehme, F. W. (2000). Microbial resistance to metals in the environment. Ecotoxicology and Environmental Safety, 45, 198-207.

Cakmak, G., Togan, I., Severcan, F. (2006). 17 Beta-Estradiol induced compositional, structural and functional changes in rainbow trout liver, revealed by FT-IR spectroscopy: a comparative study with nonylphenol. Aquatic Toxicology, 77, 53–63.

Carpenter, J. F., Crowe, J. H. (1989). An infrared spectroscopic study of the interactions of carbohydrates with dried proteins. Biochemistry, 28, 3916–3922.

Casal, H. L., Mantsch, H. H. (1984). Polymorphic phase behavior of phospholipid membranes studied by Infrared spectroscopy. Biochimica and Biophysica Acta, 779, 381-401.

Chakravarty, R., Banerjee, P. C. (2012). Mechanism of Cadmium Binding on the Cell Wall of an Acidophilic Bacterium. Bioresource Technology, 108, 176-183.

Chittock, R. S., Ward, S., Wilkinson, A. S., Caspers, P., Mensch, B., Page, M. G. P., Wharton, C. W. (1999). Hydrogen bonding and protein perturbation in β-lactam acyl-enzymes of *Streptococcus pneumoniae* penicillin-binding protein PBP2x. Biochemical Journal, 338, 153-159.

Choudhary, S., Sar, P. (2009). Characterization of a metal resistant *Pseudomonas* sp. isolated from uranium mine for its potential in heavy metal (Ni^{2+}, Co^{2+}, Cu^{2+}, and Cd^{2+}) sequestration. Bioresource Technology, 100, 2482–2492.

Ci, Y. X., Gao, T. Y., Feng, J., Guo, Z. Q. (1999). FT-IR spectroscopic characterization of human breast tissue: implications for breast cancer diagnosis. Applied Spectroscopy, 53, 312–315.

Colthup, N. B., Daly, L. H. Wiberley, S. E. (1975). Introduction to Infrared and Raman Spectroscopy, 2nd ed.Academic Press, New York.

de Breij, A., Dijkshoorn, L., Lagendijk, E, van der Meer, J., Koster, A., Bloemberg, G., Wolterbeek, R., van den Broek, P., Nibbering, P. (2010). Do biofilm formation and interactions with human cells explain the clinical success of Acinetobacter baumannii? PLoS One, 5 (5), e10732.

Denich, D. J., Beaudette, L. A., Lee, H., Trevors, J. T. (2003). Effect of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. Journal of Microbiological Methods, 52, 149-182.

Denich, T. J., Beaudette, L. A., Lee, H., Trevors, J. T. (2003). Effects of selected environmental and physico-chemical factors on bacterial cytoplasmic membranes. Journal of Microbiological Methods, 52, 149-182.

Deratani, A., Sebille, B. (1981) Metal ion extraction with a thiol hydrophilic resin. Analytical Chemistry, 53, 1742–1746.

Dziuba B., Babuchowski, A., Nalecz, D., Niklewicz, M. (2007). Identification of lactic acid bacteria using FTIR spectroscopy and cluster analysis. International Dairy Journal, 17, 183–189.

Eisler, R. (1996). Silver hazards to fish, wildlife, and invertebrates: a synoptic review. Contaminant Hazard Reviews, Biological Report 32, National Biological Service, Washington, DC, 1–44.

Euzeby, J. P. (1997). List of Bacterial Names with Standing in Nomenclature: a folder available on the Internet. International Journal of Systematic Bacteriology, 47, 590–592.

Falagas, M. E., Karveli, E. A., Kelesidis, I., Kelesidis, T. (2007). Community-acquired Acinetobacter infections. European Journal of Clinical Microbiology and Infectious Diseases, 26, 857-868.

Feng, Q. L, Wu, J., Cheng, G. Q., Cui, F. Z., Kim, T. N., Kim, J. O. (2000). A mechanistic study of the antibacterial effect of silver ions on *Escherichia coli* and *Staphylococcus aureus*. Journal of Biomedical Materials Research, 52, 662–668.

Feo, J. C., Castro, M. A., Robles, L. C., Aller, A. J. (2004). Fourier-transform infrared spectroscopic study of the interactions of selenium species with living bacterial cells. Analytical and Bioanalytical Chemistry, 378, 1601-1607.

Gao, X. D., Chorover, J. (2009). In-situ monitoring of Cryptosporidium parvum oocystsurface adhesion using ATR-FTIR spectroscopy. Colloids and Surfaces B: Biointerfaces, 71, 169–176.

Garip, S., Gozen, A. G., Severcan, F. (2009). Use of Fourier transform infrared spectroscopy for rapid comparative analysis of *Bacillus and Micrococcus* isolates. Food Chemistry, 113, 1301-1307.

George, L., Sankaran, K., Viswanathan, K. S., Mathews, C. K. (1994). Matrix isolation infrared spectroscopy of hydrogen-bonded complexes of triethyl phosphate with H_2O, DO, and methanol. Applied Spectroscopy, 48, 801–807.

Ghandour, W., Hubbard, J. A., Deistung, J., Hughes, M.N., Poole, R.K. (1988). The uptake of silver ions by *Escherichia coli* K12: toxic effects and interaction with copper ions. Applied Microbiology and Biotechnology, 28, 559-565.

Giamarellou, H., Antoniadou, A., Kanellakopoulou, K. (2008). *Acinetobacter baumannii*: a universal threat to public health? International Journal Antimicrobial Agents, 32, 106-119.

Goormaghtigh, E., Cabiaux, V., Ruysschaert, J. M. (1994). Determination of soluble and membrane protein structure by Fourier transform infrared spectroscopy. Chapter III- Secondary structures. Subcellular Biochemistry, 23, 405-450.

Gootz, T. D., Marra, A. (2008). *Acinetobacter baumannii*: an emerging multidrugresistant threat. Expert Review of Anti-Infective Therapy, 6, 309–325.

Gorgulu, S. T., Dogan, M., Severcan, F. (2007). The characterization and differentiation of higher plants by Fourier transform infrared spectroscopy. Applied Spectroscopy, 61, 300-308.

Grogan, D. W., Cronan, J. E. Jr. (1997). Cyclopropane ring formation in membrane lipids of bacteria. Microbiology and Molecular Biology Reviews, 61 (4), 429-441.

Grotiuz, G., Sirok, A., Gadea, P., Varela, G., Schelotto, F. (2006). Shiga toxin 2-producing *Acinetobacter haemolyticus* associated with a case of bloody diarrhea. Journal of Clinical Microbiology, 44, 3838–3841.

Guschina, I. A., Harwood, J. L. (2006). Lead and copper effects on lipid metabolism in cultured lichen photobionts with different phosphorus status. Phytochemistry, 67, 1731-1739.

Guzzo, A., DuBow, M. S. (1994). Identification and characterization of genetically programmed responses to toxic metal exposure in *Escherichia coli*. FEMS Microbiology Reviews, 14, 369-374.

Hadden, J. M., Bloemendal, M., Haris, P. I., Srai, S. K. S., Chapman, D. (1994). Structure and Thermal Comparison of Human Transferrin, Rabbit Serum Transferrin and Human Lactoferrin by FTIR Spectroscopy. Biochemica and Biophysica Acta, 1209, 29-36.

Hajialigol, S., Taher, M. A., Malekpour, A. (2006). A New Method for Selective Removal of Cadmium and Zinc by Modified Clinoptilolite. Journal of Adsorption Science, 24 (6), 487-496.

Haris, P. I., Chapman, D. (1992). Does Fourier Transform Infrared-Spectroscopy provide useful information on protein structures? Trends in Biochemical Sciences, 17, 328-333.

Haris, P. I., Lee, D. C., Chapman, D. (1986). A Fourier transform infrared investigation of the structural differences between ribonuclease A and Ribonuclease S. Biochimica and Biophysica Acta, 874, 255-265.

Haris, P. I., Severcan, F. (1999). FTIR spectroscopic characterization of protein structure in aqueous and non-aqueous media. Journal of Molecular Catalysis B: Enzymatic, 7, 207–221.

Hartwig, A., Asmuss, M., Ehleben, I., Herzer, U., Kostelac, D., Pelzer, A., Schwerdtle, T., Bürkle, A. (2002). Interference by Toxic Metal Ions with DNA Repair Processes and Cell Cycle Control: Molecular Mechanisms. Environmental Health Perspectives, 110- 5, 797–799.

Hassen, A., Saidi, N., Chérıf, M., Boudabous, A. (1998). Effects of heavy metals on *Pseudomonas aeruginosa* and *Bacillus thuringiensis*. Bioresource Technology, 65, 73-82.

Heipieper, H. J., Meinhardt, F., Segura, A. (2003). The cis-trans isomerase of unsaturated fatty acids in *Pseudomonas* and *Vibrio*: biochemistry, molecular biology and physiological function of a unique stress adaptive mechanism. FEMS Microbiology Letters, 229 (1), 1-7.

Helm, D., Naumann, D. (1995). Identification of some bacterial cells components by FT-IR spectroscopy. FEMS Microbiology Letters, 126, 75–80.

Holm, O., Hansen, E., Lassen, C., Stuer-Lauridsen, F., Kjolholt, J. (2002). European Commission DG ENV. E3, HeavyMetals in Waste, Final Report, COWI Consulting Engineers, Denmark.

Hoostal, M. J., Bidart-Bouzat, M. G., Bouzat, J. L. (2008). Local adaptation of microbial communities to heavy metal stress in polluted sediments of Lake Erie. FEMS Microbiology Ecology, 65, 156-168.

Hoyle, B., Beveridge, T. S. (1983). Binding of metallic ions to the outer membrane of *Escherichia coli*. Applied and Environmental Microbiology, 46, 749-752.

Hu, J., Robinson, J. L. (2010). Systematic review of invasive Acinetobacter infections in children. Canadian Journal of Infectious Diseases Medical and Microbiology, 21, 83-88.

Huang, F., Dang, Z., Guo, C. L., Lu, G. N., Gu, R. R., Liu, H. J., Zhang, H. (2013). Biosorption of Cd (II) by live and dead cells of *Bacillus cereus* RC-1 isolated from cadmium-contaminated soil. Colloids and Surfaces B: Biointerfaces, 107, 11– 18.

Huang, W., Liu, Z. (2013). Biosorption of Cd(II)/Pb(II) from aqueous solution by biosurfactant-producing bacteria: Isotherm kinetic characteristic and mechanism studies. Colloids and Surfaces B: Biointerfaces, 105, 113– 119.

Hughes, M. N., Poole, R. K. (1989). Metals and Micro-or*g*anism, 280-285, Chapman and Hall, London.

Jackson, M., Haris, P. I., Chapman, D. (1991). Fourier transform infrared spectroscopic studies of Ca^{2+} -binding proteins. Biochemistry, 30, 9681-9686.

Jansen, E., Michels, M., Til, M., Doelman, P. (1994). Effects of heavy metals in soil on microbial diversity and activity as shown by the sensitivity-resistance index, an ecologically relevant parameter. Biology and Fertility of Soils, 17, 177-184.

Jarup, L. (2003). Hazards of heavy metal contamination. British Medical Bulletin, 68, 167-182.

Ji, G., Silver, S. (1995). Bacterial resistance mechanism for heavy metals of environmental concern. Journal of Industrial Microbiology, 14, 61-75.

Jiang, W., Fan, W. (2008). Bioremediation of heavy metal-contaminated soils by sulfate-reducing bacteria. Annals of New York Academic Science, 1140, 446-454.

Jiang, W., Saxena, A., Song, B., Ward, B. B., Beveridge, T. J., Myneni, S. C. B. (2004). Elucidation of functional groups on gram-Positive and gram-Negative bacterial surfaces using infrared spectroscopy. Langmuir, 20, 11433–11442.

Joly-Guillou, M. L. (2005). Clinical impact and pathogenicity of Acinetobacter. Clinical microbiology and infection the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 11, 868-873.

Jung, J., Noh, J., Park, W. (2011). Physiological and metabolic responses for hexadecane degradation in *Acinetobacter oleivorans* DR1. Journal Microbiology, 49, 208–215.

Kamnev, A. A., Antonyuk, L. P., Matora, L. Y., Serebrennikova, O. B., Sumaroka, M. V., Colina, M., Renou-Gonnord, M. F., Ignatov, V. V. (1999). Spectroscopic characterization of cell membranes and their constituents of the plant-associated soil bacterium *Azospirillum brasilense*. Journal of Molecular Structure, 481, 387-393.

Kardas, M. Gozen, A. G., Severcan, F. (2014). FTIR spectroscopy offers hints towards widespread molecular changes in cobalt-acclimated freshwater bacteria. Aquatic Toxicology, 155, 15-23.

Kazy, S. K., D'Souza, S. F., Sar, P. (2009). Uranium and thorium sequestration by a *Pseudomonas* sp.: mechanism and chemical characterization. Journal of Hazardous, 163 (1), 65-72.

Kazy, S. K., Das, S. K., Sar, P. (2006). Lanthanum biosorption by a *Pseudomonas* sp. equilibrium studies and chemical characterization. Journal of Industrial Microbiology and Biotechnology, 33, 773–783.

Keen, E. F. 3rd, Robinson, B. J., Hospenthal, D. R., Aldous, W. K., Wolf, S.E., Chung, K. K., Murray, C. K. (2010). Prevalence of multidrug-resistant organisms recovered at a military burn center. Burns, 36, 819–825.

Kidambi, S. P., Sundin, G. W., Palmer, D. A., Chakrabarty, A. M., Bender C. L. (1995). Copper as a signal for alginate synthesis in *Pseudomonas syringae* pv. *syringae*. Applied and Environmental Microbiology, 61, 2172–2179.

Kim, I. S., Lee, H., Trevors, J. T. (2001). Effects of 2,2V,5,5V-tetrachlorbiphenyl and biphenyl on cell membranes of *Ralstonia eutropha* H850. FEMS Microbiology Letters, 200, 17– 24.

Kim, T. N., Feng, Q. L., Kim, J. O., Wu, J., Wang, H., Chen, G. C. and Cui, F. Z. (1998) . Antimicrobial effects of metal ions (Ag, Cu, Zn) in hydroxyapatite. Journal of Materials Science: Materials in Medicine, 9, 129-134.

Korkmaz, F., Severcan, F. (2005). Effect of progesterone on DPPC membrane: Evidence for lateral phase separation and inverse action in lipid dynamics. Archives of Biochemistry and Biophysics, 440, 141-147.

Lamprell, H., Mazerolles, G., Kodjo, A., Chamba, J. F., Noel, Y., Beuvier, E. (2006). Discrimination of *Staphylococcus aureus* strains from different species of *Staphylococcus* using Fourier transform infrared (FTIR) spectroscopy. International Journal of Food Microbiology, 108, 125-129.

Lebrun, M., Andurier, A., Cossart, P. (1994). Plasmid-borne cadmium resistance genes in *Listeria monocytogenes* are present on Tn5422, a novel transposon closely related to Tn917. Journal of Bacteriology, 176, 3049-3061.

Liquier, J., Akhebat, A., Taillandier, E., Ceolin, F., Huynh-Dinh, T., Igolen, J. (1991). Characterization by FTIR spectroscopyof the oligoribonucleotide duplexes r(A-U)and r(A-U) , Spectrochimica Acta, 47A, 177–186.

Luckarift, H. R., Sizemore, S. R., Farrington, K. E., Fulmer, P. A., Biffinger, J. C., Nadeau, L. J., Johnson, G. R. (2011). Biodegradation of medium chain hydrocarbons by *Acinetobacter venetianus* 2AW immobilized to hair-based adsorbent mats. Biotechnology Progress, 27 (6), 1580-1587.

Luna, C. M., Aruj, P. K. (2007). Nosocomial *Acinetobacter pneumonia*. Respirology, 12, 787–791.

Marcelli, A., Cricenti, A., Kwiatek W. M., Petibois, C. (2012). Biological applications of synchrotron radiation infrared spectromicroscopy. Biotechnology Advances, 30, 1390–1404.

Markowicz, A., Plociniczak, T., Piotrowska-Seget Z. (2010). Response of bacteria to heavy metals measured as changes in FAME profiles. Polish Journal of Environmental Studies, 19 (5), 957-965.

Martinez, C. E., Asensio, T. M., Blanco, R. V. M., Suarez, R. M. L., Torrico, M. A., Llosa, C. A. (1995). Infective endocarditis of an interventricular patch caused by *Acinetobacter haemolyticus*. Infection, 23, 243-245.

Martinez, R. J., Wang, Y., Raimondo, M. A., Coombs, J. M., Barkay, T., Sobecky, P. A. (2006). Horizontal gene transfer of PIB-type ATPases among bacteria isolated from radionuclide- and metal-contaminated subsurface soils. Applied Environmental Microbiology, 72, 3111-3118.

Martins, R. J. E., Pardo, R., Boaventura, R. A. R. (2004). Cadmium (II) and zinc (II) adsorption by the aquatic moss *Fontinalis antipyretica*: effect of temperature, pH and water hardness, Water Research, 38, 693-699.

Mathlouthi, M., Koenig, J. L. (1986). Vibrational spectra of carbohydrates. Advances in Carbohydrate Chemistry and Biochemistry, 44, 7-89.

Matlock, M. M., Henke, K. R., Atwood, D. A. (2002). Effectiveness of commercial reagents for heavy metal removal from water with new insights for future chelate designs. Journal of Hazardous Materials, 92, 129-142.

McEntee, J. D., Woodrow, J. R., Quirk, A. V. (1986). Investigation of cadmium resistance in *Alcaligenes* sp. Applied and Environmental Microbiology, 51, 515-520.

Mecozzi, M., Pietroletti, M., Di Mento, R. (2007). Application of FTIR spectroscopy in ecotoxicological studies supported by multivariate analysis and 2D correlation spectroscopy. Vibrational Spectroscopy, 44, 228-235.

Melin, A. M., Perromat, A., Deleris, G. (2001). Effect of radical attack on bacteria: an application of FT-IR spectroscopy. Applied Spectroscopy, 55, 23–28.

Mergeay, M. (1991). Towards an understanding of the genetics of bacterial metal resistance. Trends in Biotechnology, 9, 17-24.

Mitra, R. S., Gray, R. I. L., Chin, B., Bernstein, L. A. (1975). Molecular mechanims of accommodation in *Echerichia coli* to toxic levels of Cd. Journal of Bacteriology, 121, 1180-1188.

Monteiro, D. R., Gorup, L. F., Takamiya, A. S., Ruvollo-Filho, A. C., de Camargo, E. R. and Barbosa D. B. (2009). The growing importance of materials that prevent microbial adhesion: antimicrobial effect of medical devices containing silver. International Journal of Antimicrobial Agents, 34, 103-110.

Moreira Silva, G., Morais L., Marques, L., Senra, V. (2012). Acinetobacter community-acquired pneumonia in a healthy child. Revista Portuguesa De Pneumologia 18: 96–98.

Morones, J. R., Elechiguerra, J. L., Camacho, A., Holt, K., Kouri, J. B., Ramírez, J. T., Yacaman, M. J. (2005). The bactericidal effect of silver nanoparticles. Nanotechnology, 16 (10), 2346-2353.

Mujumdar, S. S., Bashetti, S. P., Chopade, B. A. (2014). Plasmid pUPI126-encoded pyrrolnitrin production by *Acinetobacter haemolyticusA19* isolated from the rhizosphere of wheat. World Journal of Microbiology and Biotechnology, 30,495-505.

Naik, M. M., Dubey, S. K. (2013). Lead resistant bacteria: Lead resistance mechanisms, their applications in lead bioremediation and biomonitoring. Ecotoxicology and Environmental Safety 98, 1–7.

Naumann, D. (1984). Some ultrastructural information on intact, living bacterial cells and related cell-wall fragments as given by FTIR. Infrared Physics, 24, 233-238.

Naumann, D. (2001). FT- Infrared and FT-Raman Spectroscopy in biomedical research. Applied Spectroscopy Reviews, 36, 239-298.

Naumann, D., Schultz, C. P., Helm, D. (1996). What can infrared spectroscopy tell us about the structure and composition of intact bacterial cells? In Infrared Spectroscopy of Biomolecules (Mantsch, H.H. and Chapman, D., eds), 279–310.

Nichols, P. D., Henson, J. M., Guckert, J. B., Nivens, D. E., White, D. C. (1985). Fourier transform-infrared spectroscopic methods for microbial ecology: analysis of bacteria, bacteria-polymer mixtures and biofilms. Journal of Microbiological methods, 4, 79-94.

Nieboer, E., Fletcher, G. G. (1996). Determinants of reactivity in metal toxicology. Toxicology of Metals, 113–132.

Nies, D. H. (1992). Resistance to cadmium, cobalt, zinc, and nickel in microbes. Plasmid, 27, 17-28.

Nies, D. H. (1999). Microbial heavy-metal resistance. Applied Microbiology and Biotechnology, 51, 730-750.

Nies, D. H., Silver, S. (1995). Ion efflux systems involved in bacterial metal resistances. Journal of Industrial Microbiology and Biotechnology, 14, 189-199.

Nithya, C., Gnanalakshmi, B., Pandian S. K. (2011). Assessment and characterization of heavy metal resistance in Palk Bay sediment bacteria. Marine Environmental Research, 71, 283-294.

Ong, C. W. M., Lye, D. C. B., Khoo, K.L., Chua, G. S. W., Yeoh, S. F., Leo, Y. S., Tambyah, P. A., Chua, A. C. (2009). Severe community-acquired *Acinetobacter baumannii* pneumonia: an emerging highly lethal infectious disease in the Asia-Pacific. Respirology, 14, 1200–1205.

Ozaki, T., Nishimura, N., Arakawa, Y., Suzuki, M., Narita, A., Yamamoto, Y., Koyama, N., Nakane, K., Yasuda, N., Funahashi, K. (2009). Community-acquired *Acinetobacter baumannii* meningitis in a previously healthy 14-month-old boy. Journal of Infection Chemotherapy, 15, 322–324.

Ozaktas, T., Taskin, B., Gozen A. G. (2012). High level multiple antibiotic resistance among fish surface associated bacterial populations in non-aquaculture freshwater environment. Water Research, 46 (19), 6382-6390.

Ozek, N. S., Bal, B. Yıldırım, S. Onur, S., Severcan, S. (2014). Structural and functional characterization of simvastatin-induced myotoxicity in different skeletal muscles. Biochemica and Biophysica Acta, 1840, 406-415.

Pages, D., Sanchez, L., Conrod, S., Gidrol, X., Fekete, A., Schmitt-Kopplin, P., Heulin, T., Achouak, W. (2007). Exploration of intraclonal adaptation mechanisms of *Pseudomonas brassicacearum* facing cadmium toxicity. Environmental Microbiology, 9 (11), 2820–2835.

Pagnanelli, F., Peterangelipapin, M., Toro, I. L., Trifoni, M., Veglio, F. (2000). Biosorption of metal ions on *Arthrobacter* sp.: biomass characterization and biosorption modeling. Environmental Science and Technology, 34, 2773–2778.

Pandey, A., Naik, M. M., Dubey, S. K. (2011). Biological characterization of marine fish pathogen, *Acinetobacter sp*. strain An 2 producing antibacterial metabolites. Journal of Scientific and Industrial Research, 70, 135-141.

Paul, M. L., Samuel, J., Chandrasekaran, N., Mukherjee, A. (2012). Comparative kinetics, equilibrium, thermodynamic and mechanistic studies on biosorption of hexavalent chromium by live and heat killed biomass of *Acinetobacter junii* VITSUKMW2, an indigenous chromite mine isolate. Chemical Engineering Journal, 187, 104–113.

Peleg, A. Y., Seifert, H., Paterson, D. L. (2008). *Acinetobacter baumannii*: emergence of a successful pathogen. Clinical Microbiology Reviews, 21, 538-582.

Pohle, W., Bohl, M., Böhlig, H. (1990). Interpretation of the influence of hydrogen bonding on the stretching vibrations of the PO_2 moiety. Journal of Molecular Structure, 242, 333-342.

Pohle, W., Fritzsche, H. (1980). A new conformation-specific infrared band of A-DNA in films. Nucleic Acids Research, 8, 2527–2535.

Poole, R. K., Gadd, G. M. (1989). Metals: Microbe Interactions, 1-37, IRL Press, Oxford.

Quilès, F., François, H., Delille, A. (2010). Analysis of changes in attenuated total reflection FTIR fingerprints of *Pseudomonas fluorescens* from planktonic state to nascent biofilm state. Spectrochimica Acta Part A: Molecular and Biomoleculer Spectroscopy, 75, 610-616.

Rathinavelu, S., Zavros, Y., Merchant, J. L. (2003). *Acinetobacter lwoffii* infection and gastritis. Microbes and Infections, 5, 651-657.

Regalado, N. G., Martin, G., Antony, S. J. (2009). *Acinetobacter lwoffii*: bacteremia associated with acute gastroenteritis. Travel Medicine and Infectious Disease, 7, 316–317.

Rigas, B., Morgello, S., Goldman, I. S., Wong, P. T. (1990). Human colorectal cancers display abnormal Fourier-transform infrared spectra. Proceedings of the National Academy of Sciences, 87, 8140-8144.

Roane, T. M. (1999). Lead Resistance in Two Bacterial Isolates from Heavy Metal-Contaminated Soils. Microbial Ecology, 37, 218-224.

Rosenman, K. D., Moss, A., Kon, S. (1979). Argyria: clinical implications of exposure to silver nitrate and silver oxide. Journal of Occupational Environmental Medicine, 21, 430-435.

Rosenman, K. D., Seixas, N., Jocobs, I. (1987). Potential nephrotoxiceffects of exposure to silver. British Journal of Industrial Medicine, 44, 267-272.

Rouch, D. A., Lee, B. T. D., Morby, A. P. (1995). Understanding cellular responses to toxic agents: A model for mechanism choice in bacterial metal resistance. Journal of Industrial Microbiology, 14, 132-141.

Satarug, S., Baker, J. R., Urbenjapol, S., Haswell-Elkins, M., Reilly, P. E. B., Williams, D. J., Moore, M. R. (2003). A global perspective on cadmium pollution and toxicity in non-occupationally exposed population. Toxicology Letters, 137 (1-2), 65-83.

Saulou, C., Jamme, F., Girbal, L., Maranges, C., Fourquaux, I., Cocaign-Bousquet, M., Dumas, P., Mercier-Bonin, M. (2013). Synchrotron FTIR microspectroscopy of *Escherichia coli* at single-cell scale under silver-induced stress conditions. Analytical and Bioanalytical Chemistry, 405, 2685–2697.

Schreurs, W. J. A., Rosenberg, H. (1982). Effect of silver ions on transport and retention of phosphate by *Escherichia coli* . Journal of Bacteriology, 152, 7-13.

Schultz, C., Naumann, D. (1991). In vivo study of the state of order of the membranes of Gram-negative bacteria by Fourier-transform infrared spectroscopy (FT-IR). FEBS Letters, 294, 43-46.

Schuster, K. C., Mertens, F., Gapes, J. R. (1999). FTIR spectroscopy applied to bacterial cells as a novel method for monitoring complex biotechnological processes. Vibrational Spectroscopy, 19, 467–477.

Scott, J. A., Palmer, S. J. (1988). Cadmium bio-sorption by bacterial exopolysaccharide. Biotechnology Letters, 10, 21-24.

Scott, J. A., Palmer, S. J. (1990). Sites of cadmium uptake in bacteria used for biosorption. Applied and Environmental Microbiology, 33, 221-225.

Scott, J. A., Sage, G. K., Palmer, S. J. (1988). Metal immobilization by microbial capsular coatings. Biorecovery, 1, 51-58.

Selvin, J., Joseph, S., Asha, K. R., Manjusha, W. A., Sangeetha, V. S., Jayaseema, D. M., Antony, M. C., Denslin Vinitha, A. J. (2004). Antibacterial potential of antagonistic *Streptomyces* sp. isolated from marine sponge *Dendrilla nigra*. FEMS Microbiology and Ecology, 50, 117-122.

Sengstock, D. M., Thyagarajan, R., Apalara, J., Mira, A., Chopra, T., Kaye, K. S. (2010). Multidrug-resistant *Acinetobacter baumannii*: an emerging pathogen among older adults in community hospitals and nursing homes. Clinical Infectious Diseases, 50, 1611–1616.

Severcan, F. (1997). Vitamin E Decreases the Order of the Phospholipid Model Membranes in the Gel Phase: An FTIR Study. Bioscience Reports, 17, 231-235.

Severcan, F., Sahin I., Kazancı, N. (2005). Melatonin strongly interacts with zwitterionic model membranes-evidence from Fourier transform infrared spectroscopy and differential scanning calorimetry. Biochimica and Biophysica Acta, 1668, 215-222.

Siebert, F. (1995). Infrared spectroscopy applied to biochemical and biological problems. Methods in Enzymology, 246, 501–526.

Silver, S., Ji, G. (1994). Newer systems for bacterial resistance's to toxic heavy metals. Environmental Health Perspectives, 102, 107-113.

Silver, S., Walderhaug, M., (1992). Gene regulation and chromosome determined inorganic ion transport in bacteria. Microbiological Reviews, 56, 195-228.

Silvestry-Rodriguez, N., Bright, K. R., Slack, D. C, Uhlmann, D. R., Gerba, C. P. (2008). Silver as a residual disinfectant to prevent biofilm formation in water distribution systems. Applied and Environmental Microbiology, 74, 1639–1641.

Simoes, L. C., Simoes, M., Vieira, M. J. (2008). Intergeneric coaggregation among drinking water bacteria: evidence of a role for *Acinetobacter calcoaceticus* as a bridging bacterium. Applied Environmental Microbiology, 74, 1259–1263.

Simon, S. (2003). Bacterial silver resistance: molecular biology and uses and misuses of silver compounds. FEMS Microbiology Reviews, 27, 341-353.

Singh, G. B., Gupta, S., Srivastava, S., Gupta, N. (2011). Biodegradation of Carbazole by Newly Isolated *Acinetobacter* spp. Bulletin of Environmental Contamination and Toxicology, 87, 522–526.

Sockalingum, G. D., Bouhedja, W., Pina, P., Allouch, P., Mandray, C., Labia, R., Millot, J. M., Manfait M. (1997). ATR–FTIR Spectroscopic Investigation of imipenem-susceptible and -resistant *Pseudomonas aeruginosa* isogenic Strains. Biochemical and Biophysical Research Communications, 232, 240-246.

Surewicz, W. K., Mantsch, H. H., Chapman, D. (1993). Determination of protein structure by Fourier transform infrared spectroscopy: a critical assessment. Biochemistry, 32 (2), 389-394.

Susi, H., Byler, D. M. (1986). Resolution-enhanced Fourier transform infrared spectroscopy of enzymes. Methods in Enzymology, 150, 290-311.

Taillandier, E., Liquier, J. (1992). Infrared spectroscopy of DNA. Methods in Enzymology, 211, 307–335.

Tamm, L. K., Tatulian, S. A. (1997). Infrared spectroscopy of protein and peptides in lipid bilayers. Quarterly Reviews in Biophysics, 30, 365-429.

Tayabali, A. F., Nguyen, K. C., Shwed, P. S., Crosthwait, J., Coleman, G. and Seligy, V. L. (2012). Comparison of the Virulence Potential of Acinetobacter Strains from Clinical and Environmental Sources. PLoS ONE, 7(5), e37024.

Torii, H., Tasumi, M. (1992). Model-calculations on the amide-I infrared bands of globular-proteins. The Journal of Chemical Physics, 96, 3379-3387.

Trajanovska, S., Britz, M. L., Bhave, M. (1997). Detection of heavy metal ion resistance genes in gram-positive and gram-negative bacteria isolated from a lead contaminated site. Biodegradation, 8, 113-124.

Trevors, J. T., Stratton, G. W., Gadd, G. M. (1986). Cadmium transport, resistance, and toxicity in bacteria, algae, and fungi. Canadian Journal of Microbiology. 32, 447-464.

Tsuboi, M. (1969). Application of infrared spectroscopy to structure studies of nucleic acids. Applied Spectroscopy Reviews, 3, 45, 90.

van Hullebusch, E. D., Utomo, S., Zandvoort, M. H, Lens, P. N. L. (2003). Comparison of three sequential extraction procedures for the fractionation of cobalt, nickel, copper, zinc, manganese and iron in anaerobic granular sludges. Talanta, 65, 549–58.

Volker, C., Oetken, M., Oehlmann, J. (2013). The Biological Effects and Possible Modes of Action of Nanosilver. Reviews of Environmental Contamination and Toxicology, 223, 81-106.

Wang, C.L., Michels, P.C., Dawson, S.C., Kittisakkul, S., Baross, J.A., Keasling, J.D., Clark, D.S. (1997). Cadmium removal by a new strain of *Pseudomonas aeruginosa* in aerobic culture. Appl. Environ. Microbiol. 63, 4075–4078.

Wang, H., Law, N., Pearson, G., Van Dongen, B. E., Jarvis, R. M., Goodacre, R., Lloyd, J. R. (2010a),Impact of Silver(I) on the Metabolism of *Shewanella oneidensis*. Journal of Bacteriology, 192 (4), 1143–1150.

Wang, J., Kim, K. H., Sungkyun, K., Kim, Y. S., Qing, X. L., Jun, S. (2010b). Simple quantitative analysis of *Escherichia coli* K-12 internalized in baby spinach using Fourier Transform Infrared spectroscopy . International Journal of Food Microbiology, 144, 147–151.

Wang, L., Li, F., Zhou, Q. (2006). Contribution of Cell-Surface Components to Cu^{2+} Adsorption by *Pseudomonas putida 5-x,* Applied Biochemistry and Biotechnology, 128 (1), 33–46.

Wharton, C.W. (2000). Infrared spectroscopy of enzyme reaction intermediates. Natural Product Reports**,** 17, 447–453.

Wijnhoven, S. W. P., Peijnenburg W. J. G. M., Herberts, C. A., Hagens, W.I., Oomen, A. G., Heugens, E. H. W., Roszk, B., Bisschops, J., Gosens, I., Van de Meent, D., Dekkers, S., De Jong, W. H., Van Zijverden, M., Sips, A. J. A. M. Geertsma, R. E. (2009). Nano-silver a review of available data and knowledge gaps in human and environmental risk assessment. Nanotoxicology, 3, 109-138.

Wong, P. T., Wong, R. K., Caputo, T. A., Godwin, T. A., Rigas, B. (1991). Infrared spectroscopy of exfoliated human cervical cells: evidence of extensive structural changes during carcinogenesis. Proceedings of the National Academy of Sciences, 88, 10988-10992.

Wuertz, S., Spaeth, R., Hinderberger, A., Griebe, T., Flemming H. C., Wilderer, P. A. (2001). A new method for extraction of extracellular polymeric substances from biofilms and activated sludge suitable for direct quantification of sorbed metals. Water Science and Technology, 43, 25–31.

Yahya, S. K., Zakaria, Z. A., Samin, J., Santhana Raj, A. S., Ahmad, W. A. (2012). Isotherm kinetics of Cr (III) removal by non-viable cells of *Acinetobacter haemolyticus*. Colloids and Surfaces B: Biointerfaces, 94, 362–368.

Yu, C., Irudayaraj, J. (2005). Spectroscopic characterization of microorganisms by Fourier transforms infrared microspectroscopy. Biopolymers, 77, 368–377.

Zakaria, Z. A., Zakaria, Z., Surif, S., Ahmad, W. A., (2007). Hexavalent chromium reduction by *Acinetobacter haemolyticus* isolated from heavy-metal contaminated wastewater. Journal of Hazardous Materials, 146, 30–38.

Zhao, Z., Selvam, A., Wong, J. W. C. (2011). Synergistic effect of thermophilic temperature and biosurfactant produced by *Acinetobacter calcoaceticus* BU03 on the biodegradation of phenanthrene in bioslurry system. Journal Hazardous Materials, 190, 345-350.

APPENDIX A

The Significant Differences Measured by ATR-FTIR Spectroscopy after Heavy Metal Treatment for both *Acinetobacter* Strain

Table A1. The significant differences after Cd treatment for environmental *Acinetobacter* sp. (n=10)

Band no	Frequency values (cm^{-1})			
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**
9	1451.37 ± 0.29	1450.67 ± 0.54	0.05	**
10	1394.31 ± 0.50	1393.17 ± 0.95	0.08	*
16	1082.70 ± 0.26	1082.12 ± 0.40	0.05	**
18	1034.15 ± 0.49	1032.27 ± 0.67	0.18	****

Band no	Band area values (cm^{-1})			
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**
6	0.30 ± 0.02	0.16 ± 0.02	87.50	****
12	11.20 ± 0.90	8.81 ± 1.56	27.13	***
13	1.47 ± 0.16	0.68 ± 0.12	116.18	****

Band no	Bandwidth values (cm^{-1})			
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**
5	5.71 ± 0.33	5.07 ± 0.57	12.62	*
7	38.59 ± 0.65	40.31 ± 0.91	-4.27	**

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$

The "-" indicates increases and the "+" shows decreases when compared to control group values

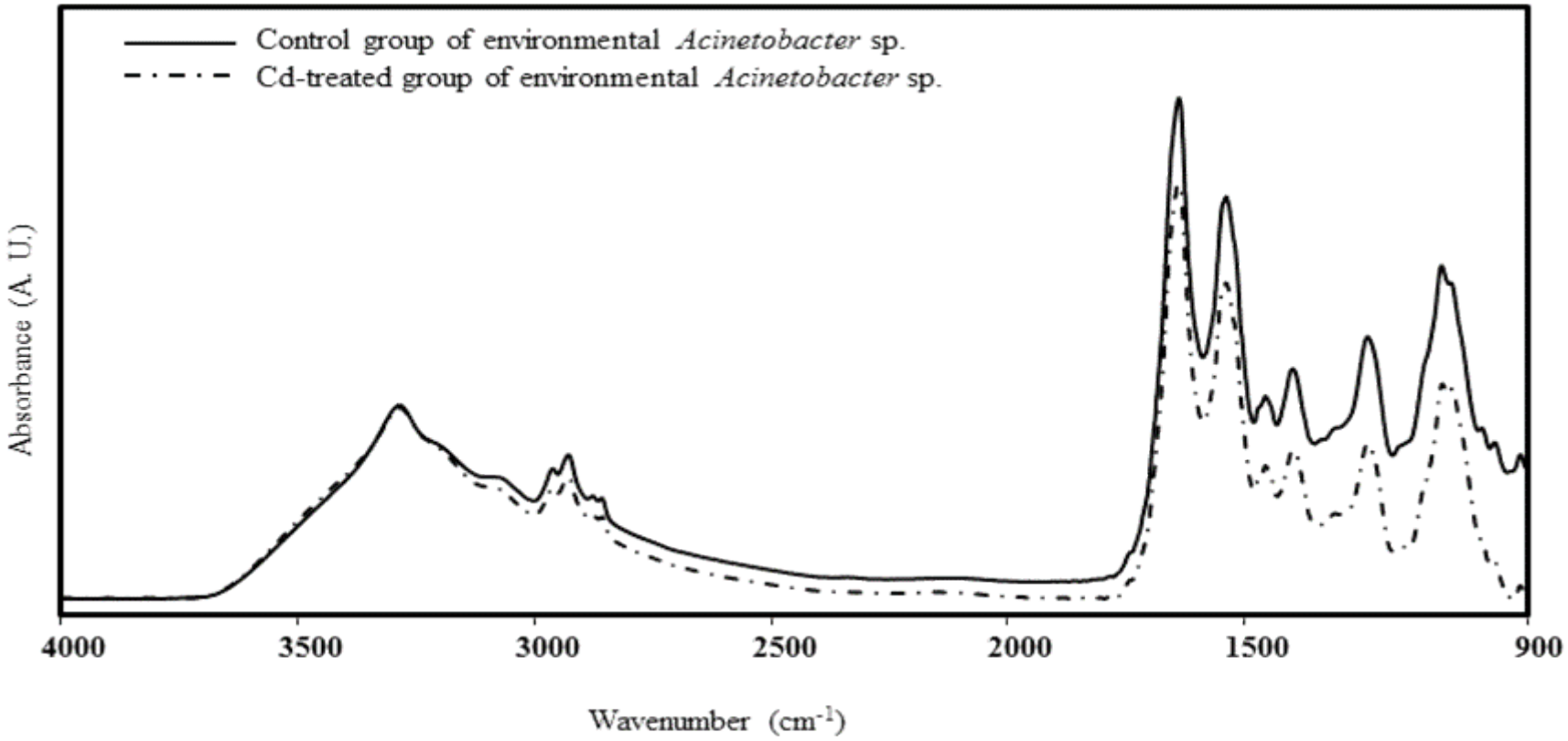

Figure A1. The average spectra of the control and Cd-treated groups of environmental *Acinetobacter* strain in the 4000-900 cm^{-1} region.

Table A2. The significant differences after Cd treatment for *A. haemolyticus* ATCC 19002 (n=10)

Band no	Frequency values (cm^{-1})			
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**
9	1450.61 ± 1.66	1448.35 ± 0.38	0.16	*
10	1392.79 ± 1.05	1394.89 ± 0.97	-0.15	****
11	1313.17 ± 1.31	1302.97 ± 1.55	0.78	****
13	1174.44 ± 0.85	1173.42 ± 0.10	0.09	**
17	1057.15 ± 0.35	1056.32 ± 0.20	0.08	****
18	1031.02 ± 0.77	1029.00 ± 0.57	0.20	****

Band no	Band area values (cm^{-1})			
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**
1	56.25 ± 7.51	46.31 ± 2.97	21.46	**
5	1.50 ± 0.11	1.37 ± 0.11	9.49	*
6	0.36 ± 0.03	0.18 ± 0.02	100.00	****
11	1.46 ± 0.25	4.09 ± 0.37	-64.30	****
19	0.91 ± 0.19	1.15 ± 0.15	-20.87	*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$
The "-" indicates increases and the "+" shows decreases when compared to control group values

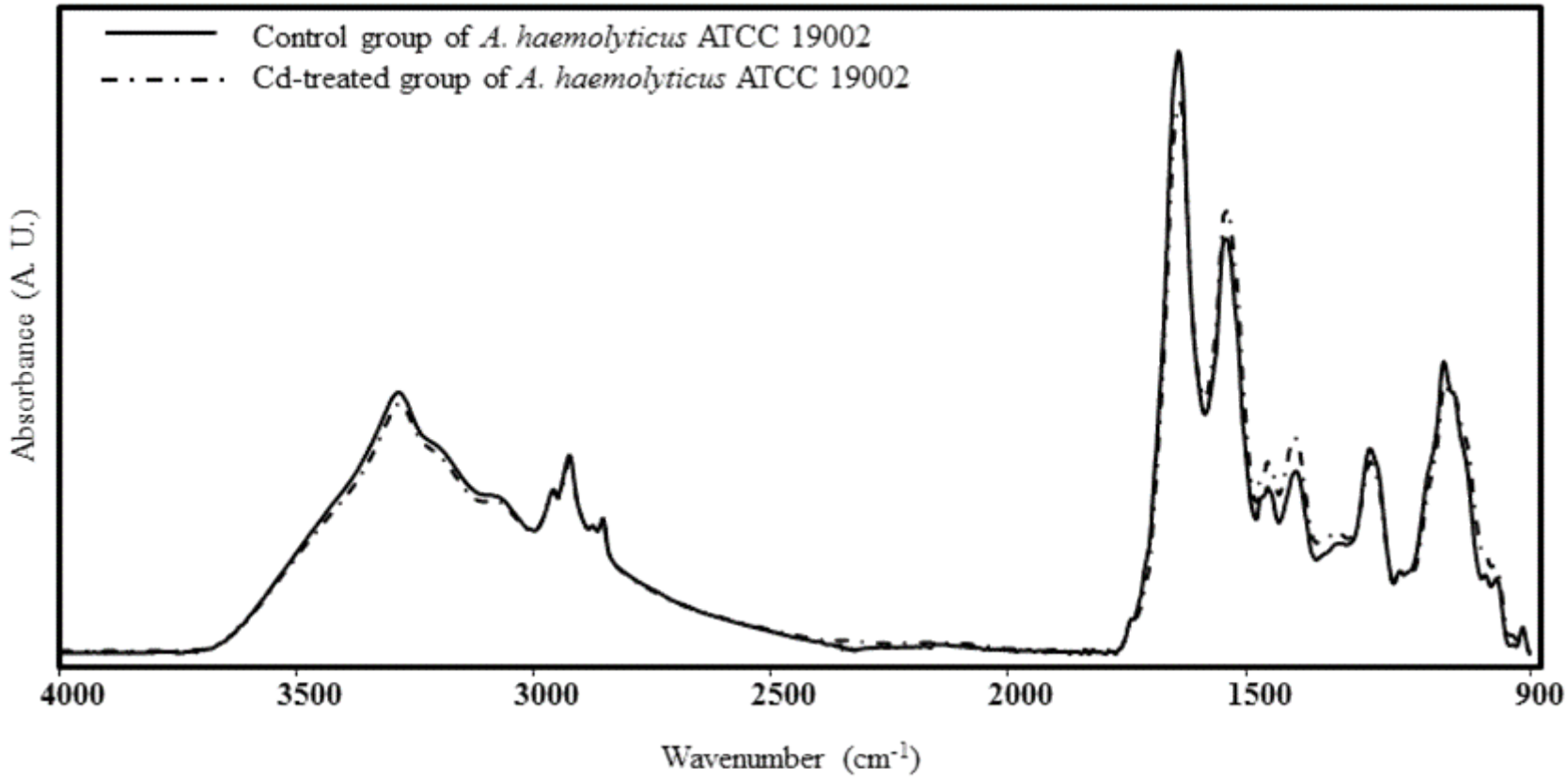

Figure A2. The average spectra of the control and Cd-treated groups of *A. haemolyticus* ATCC 19002 strain in the 4000-900 cm^{-1} region.

Table A3. The significant differences after Ag treatment for environmental *Acinetobacter* sp. (n=10)

Band no	Frequency values (cm^{-1})			
	Ctrl Mean ± SD	**Ag Mean ± SD**	**% change**	***p*-value**
4	2874.24 ± 0.42	2874.64 ± 0.19	-0.01	*
5	2852.61 ± 0.37	2851.49 ± 1.00	0.04	*
9	1451.37 ± 0.29	1450.51 ± 0.29	0.06	***
10	1394.31 ± 0.50	1392.54 ± 0.84	0.13	***
11	1308.49 ± 0.98	1311.20 ± 0.84	-0.21	**
12	1234.18 ± 0.77	1232.41 ± 1.02	0.14	**
14	NO	1161.26 ± 2.37	-	-
16	1082.70 ± 0.26	1081.97 ± 0.09	0.07	***
18	1034.15 ± 0.49	1032.45 ± 0.89	0.16	****

Band no	Band area values (cm^{-1})			
	Ctrl Mean ± SD	**Ag Mean ± SD**	**% change**	***p*-value**
1	50.04 ± 2.72	61.80 ± 6.29	-19.03	*
6	0.30 ± 0.02	0.26 ± 0.03	15.38	**
9	3.96 ± 0.26	4.86 ± 0.49	-18.52	*
11	2.30 ± 0.16	1.74 ± 0.19	32.18	*
13	1.47 ± 0.16	0.44 ± 0.07	234.09	****
14	NO	0.43 ± 0.07	-	-

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; NO, not observed
The "-" indicates increases and the "+" shows decreases when compared to control group values

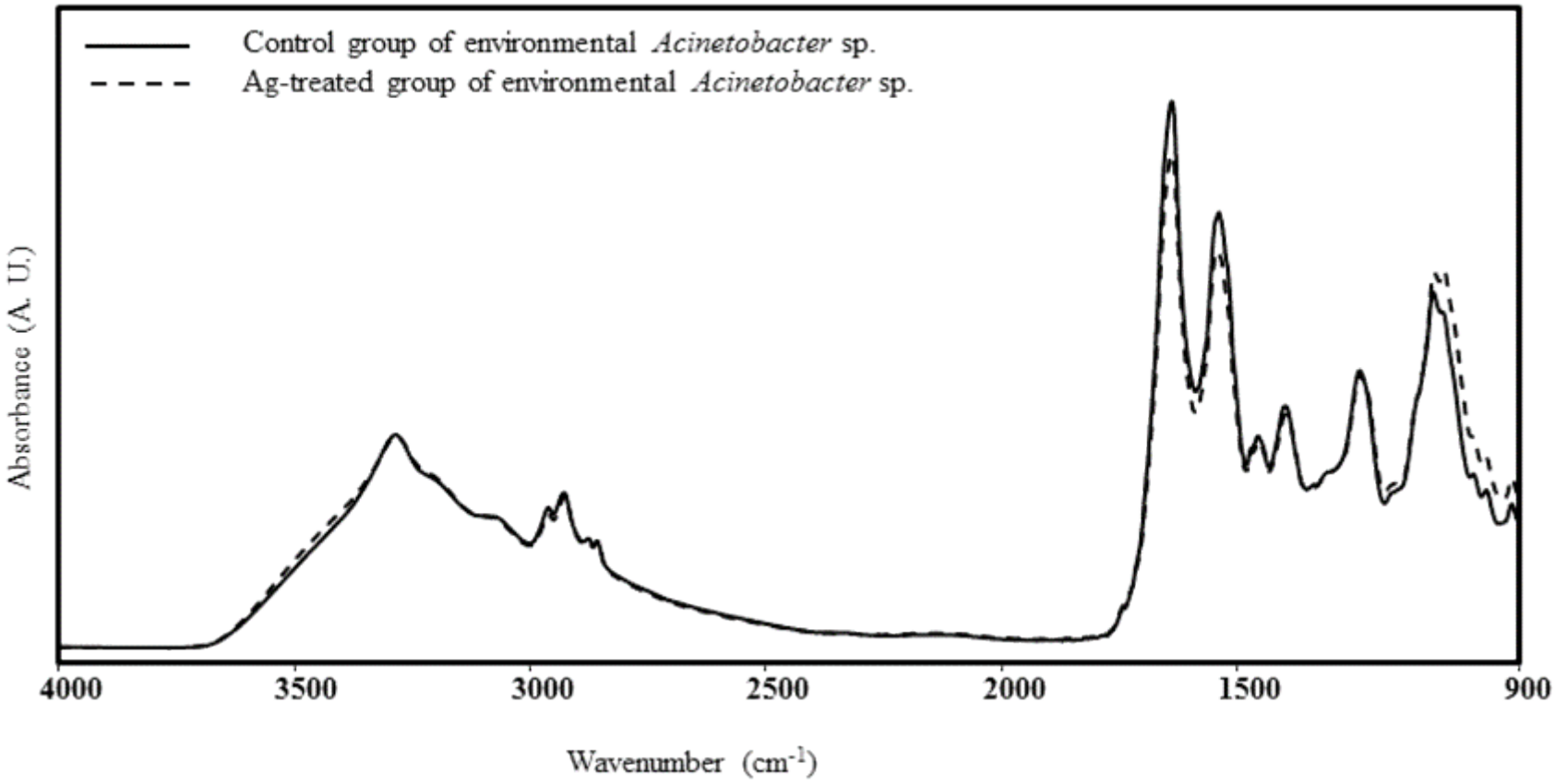

Figure A3. The average spectra of the control and Ag-treated groups of environmental *Acinetobacter* strain in the 4000-900 cm^{-1} region.

Table A4. The significant differences after Ag treatment for *A. haemolyticus* ATCC 19002 (n=10)

Band no	Frequency values (cm^{-1})			
	Ctrl Mean ± SD	**Ag Mean ± SD**	**% change**	***p*-value**
1	3263.58 ± 3.19	3271.95 ± 3.15	-0.26	****
4	2873.73 ± 0.49	2874.84 ± 0.66	-0.04	***
8	1536.53 ± 2.00	1538.17 ± 1.18	-0.11	*
11	1313.17 ± 1.31	1301.74 ± 3.09	0.88	****
12	1232.46 ± 1.33	1233.93 ± 0.96	-0.12	**
15	1116.19 ± 1.07	1113.21 ± 0.46	0.27	****
17	1057.15 ± 0.35	1056.18 ± 0.37	0.09	****
18	1031.02 ± 0.77	1028.79 ± 0.43	0.22	****

Band no	Band area values (cm^{-1})			
	Ctrl Mean ± SD	**Ag Mean ± SD**	**% change**	***p*-value**
1	56.25 ± 7.51	63.33 ± 7.25	-11.18	*
4	1.40 ± 0.13	1.18 ± 0.09	18.64	***
5	1.50 ± 0.11	1.26 ± 0.12	19.05	****
6	0.36 ± 0.03	0.27 ± 0.07	33.33	***
11	1.46 ± 0.25	0.64 ± 0.07	128.13	****
17	3.08 ± 0.61	2.45 ± 0.33	25.71	**
18	3.53 ± 0.78	2.41 ± 0.35	46.47	****
19	0.91 ± 0.19	0.58 ± 0.10	56.90	**

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$
The "-" indicates increases and the "+" shows decreases when compared to control group values

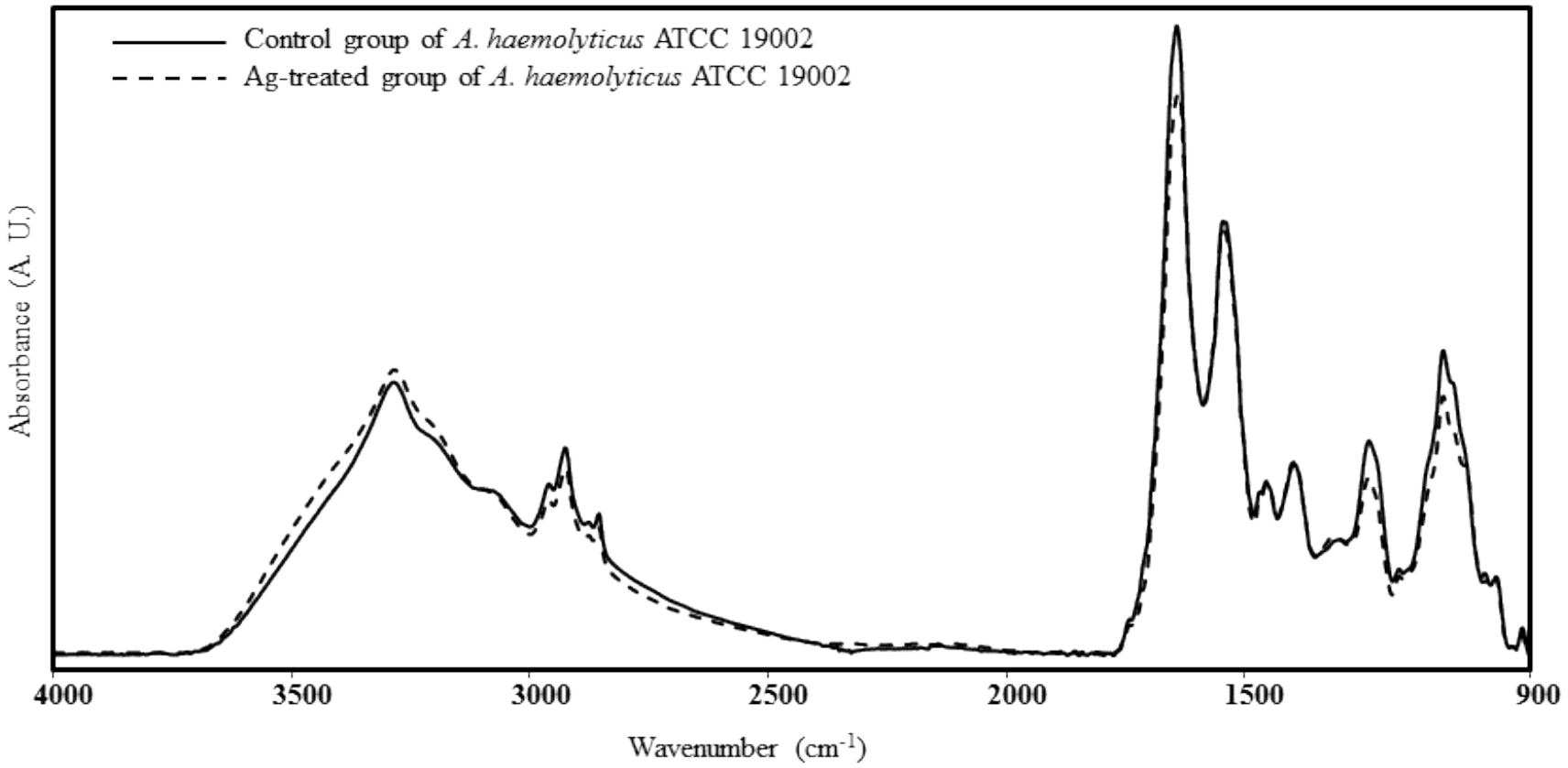

Figure A4. The average spectra of the control and Ag-treated groups of *A. haemolyticus* ATCC 19002 strain in the 4000-900 cm^{-1} region.

Table A5. The significant differences after Pb treatment for environmental *Acinetobacter* sp. (n=10)

Band no	Frequency values (cm^{-1})			
	Ctrl Mean ± SD	**Pb Mean ± SD**	**% change**	***p*-value**
1	3263.53 ± 3.59	3258.17 ± 3.31	0.16	*
4	2874.24 ± 0.42	2874.94 ± 0.35	-0.02	****
5	2852.61 ± 0.37	2851.32 ± 0.48	0.05	**
9	1451.37 ± 0.29	1449.55 ± 0.47	0.13	****
10	1394.31 ± 0.50	1392.45 ± 0.99	0.13	***
11	1308.49 ± 0.98	1311.72 ± 0.81	-0.25	***
14	-	1155.11 ± 1.42	-	-
15	1118.16 ± 0.17	1120.05 ± 1.82	-0.17	***
16	1082.70 ± 0.26	1081.34 ± 0.46	0.04	****
17	1056.46 ± 0.29	1053.57 ± 2.57	0.27	***
18	1034.15 ± 0.49	1032.22 ± 0.45	0.19	****
19	992.83 ± 0.20	978.88 ± 7.62	1.43	****
20	966.23 ± 0.27	NO	-	-

Band no	Band area values (cm^{-1})			
	Ctrl Mean ± SD	**Pb Mean ± SD**	**% change**	***p*-value**
6	0.30 ± 0.02	0.11 ± 0.02	172.73	****
7	30.01 ± 1.43	23.30 ± 5.68	28.80	**
12	11.20 ± 0.90	4.23 ± 0.70	164.78	****
13	1.47 ± 0.16	0.02 ± 0.01	7250.00	****
14	NO	2.22 ± 1.58	-	-
15	3.11 ± 0.16	1.78 ± 0.96	74.72	****
19	1.02 ± 0.09	5.83 ± 1.45	-82.50	****
20	0.64 ± 0.07	NO	-	-

Table A5. (Cont.) The significant differences after Pb treatment for environmental *Acinetobacter* sp. (n=10)

Band no	Bandwidth values (cm^{-1})			
	Ctrl Mean ± SD	**Pb Mean ± SD**	**% change**	***p*-value**
5	5.71 ± 0.33	5.05 ± 0.36	13.07	*
7	38.59 ± 0.65	44.71 ± 1.78	-13.69	****
8	37.51 ± 0.36	38.85 ± 0.68	-3.45	***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; NO, not observed
The "-" indicates increases and the "+" shows decreases when compared to control group values

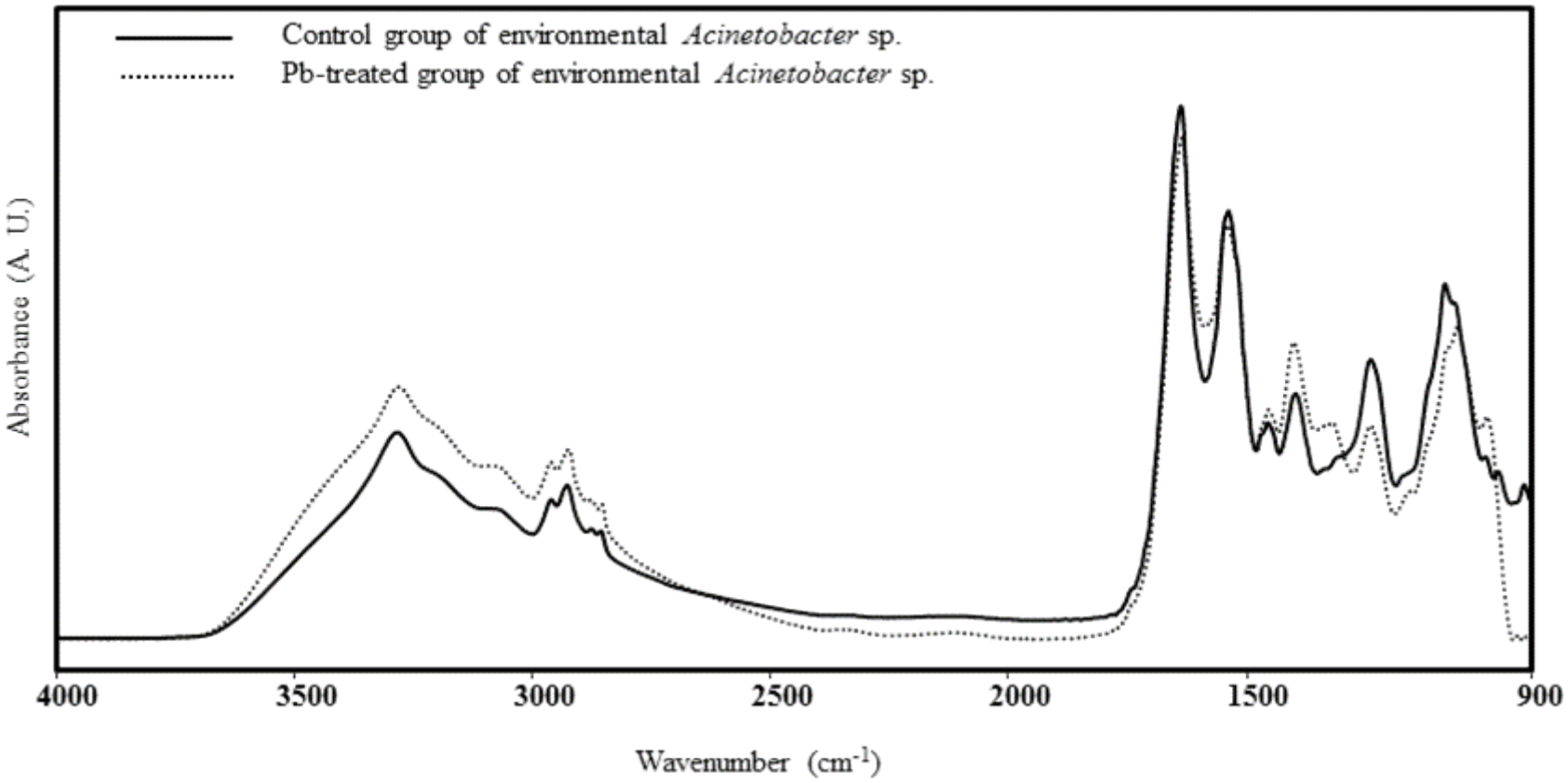

Figure A5. The average spectra of the control and Pb-treated groups of environmental *Acinetobacter* strain in the 4000-900 cm^{-1} region.

Table A6. The significant differences after Pb treatment for *A. haemolyticus* ATCC 19002 (n=10)

Band no	Frequency values (cm^{-1})			
	Ctrl Mean ± SD	**Pb Mean ± SD**	**% change**	***p*-value**
4	2873.73 ± 0.49	2874.73 ± 0.47	-0.03	***
7	1640.27 ± 1.68	1636.97 ± 0.29	0.20	****
9	1450.61 ± 1.66	1448.71 ± 0.55	0.13	**
14	1156.78 ± 0.90	1156.14 ± 0.26	0.06	*
15	1116.19 ± 1.07	1110.55 ± 1.54	0.51	****
16	1082.37 ± 0.29	1081.60 ± 0.31	0.07	****
17	1057.15 ± 0.35	1053.62 ± 0.42	0.34	****
18	1031.02 ± 0.77	1028.60 ± 0.55	0.24	****
19	991.98 ± 0.22	991.04 ± 0.26	0.09	****
20	966.04 ± 0.59	968.01 ± 1.43	-0.20	****
Band no	**Band area values (cm^{-1})**			
	Ctrl Mean ± SD	**Pb Mean ± SD**	**% change**	***p*-value**
1	56.25 ± 7.51	74.50 ± 3.95	-24.50	****
2	2.41 ± 0.23	2.79 ± 0.07	-13.62	***
3	5.89 ± 0.48	6.71 ± 0.22	-12.22	***
4	1.40 ± 0.13	1.61 ± 0.06	-13.04	***
5	1.50 ± 0.11	1.68 ± 0.07	-10.71	**
6	0.36 ± 0.03	0.23 ± 0.03	56.52	****
8	20.15 ± 3.54	23.61 ± 1.13	-14.65	**
10	7.72 ± 1.29	9.16 ± 0.63	-15.72	**
11	1.46 ± 0.25	4.67 ± 0.50	-68.74	****
12	8.33 ± 1.54	5.29 ± 0.65	57.47	****
13	1.12 ± 0.18	2.15 ± 0.18	-47.91	****
14	0.94 ± 0.15	2.10 ± 0.18	-55.24	****
15	3.42 ± 0.54	4.18 ± 0.26	-18.18	***
16	4.60 ± 0.83	7.17 ± 0.40	-35.84	****
17	3.08 ± 0.61	4.76 ± 0.23	-35.29	****
18	3.53 ± 0.78	4.51 ± 0.23	-21.73	***
19	0.91 ± 0.19	2.11 ± 0.28	-56.87	****
20	1.35 ± 0.33	2.45 ± 0.48	-44.90	****

Table A6. (Cont.) The significant differences after Pb treatment for *A. haemolyticus* ATCC 19002 (n=10)

Band no	Bandwidth values (cm^{-1})			
	Ctrl Mean ± SD	**Pb Mean ± SD**	**% change**	***p*-value**
3	18.63 ± 1.92	20.82 ± 0.65	-10.52	*

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$
The "-" indicates increases and the "+" shows decreases when compared to control group values

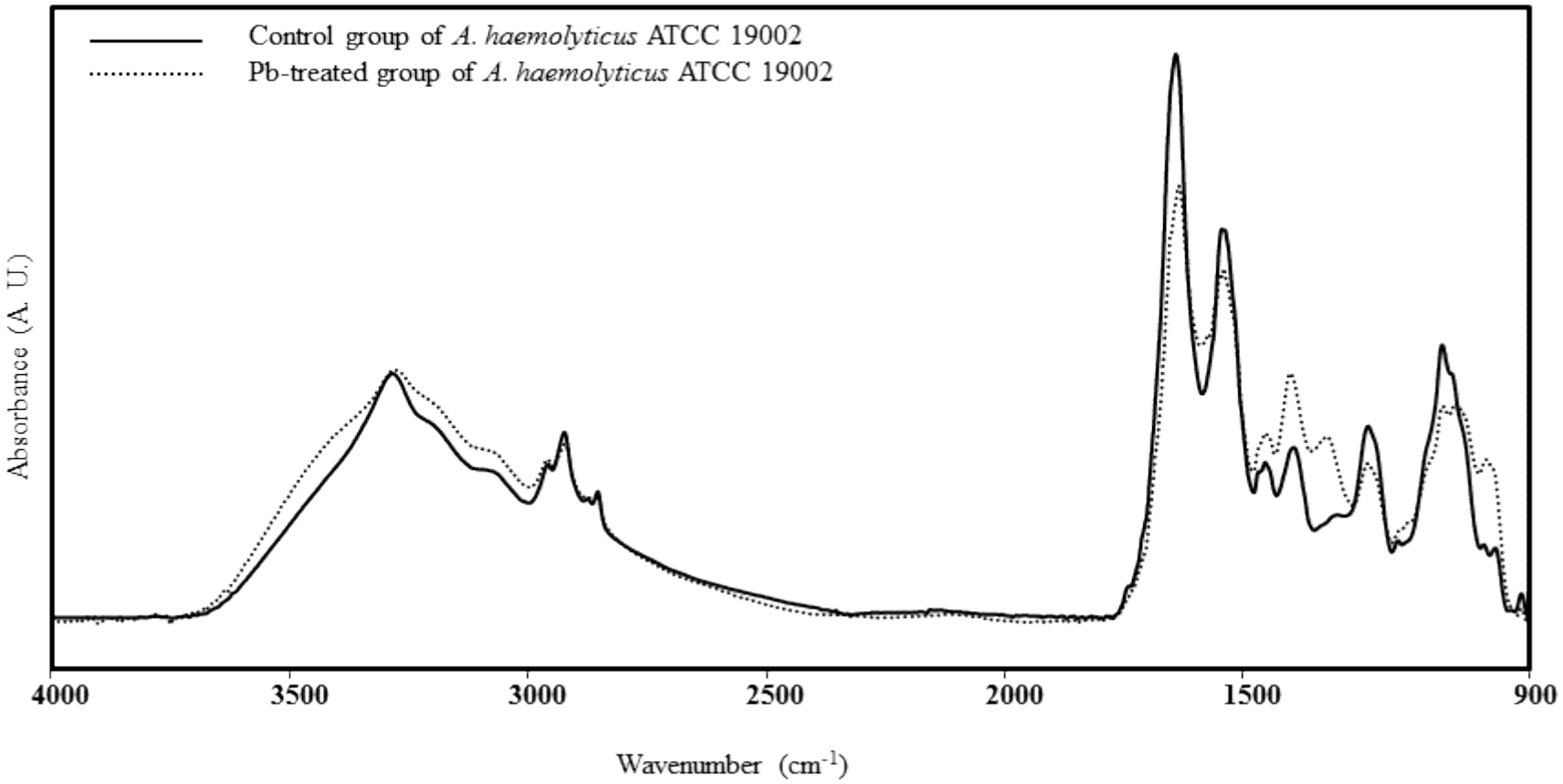

Figure A6. The average spectra of the control and Pb-treated groups of *A. haemolyticus* ATCC 19002 strain in the 4000-900 cm^{-1} region.

Table A7. The significant differences among metal treated groups for environmental *Acinetobacter* sp. (n=10)

Band no	Frequency values (cm^{-1})								
				Cd vs. Pb		Cd vs. Ag		Pb vs. Ag	
	Cd Mean ± SD	Pb Mean ± SD	Ag Mean ± SD	% change	*p*-value	% change	*p*-value	% change	*p*-value
1	3264.07 ± 5.15	3258.17 ± 3.31	3264.81 ± 3.08	0.18	**	-0.02	ns	-0.20	**
7	1639.78 ± 1.28	1637.97 ± 1.40	1639.02 ± 1.00	0.11	**	0.05	ns	-0.06	ns
9	1450.67 ± 0.54	1449.55 ± 0.47	1450.51 ± 0.29	0.08	****	0.01	ns	-0.07	****
11	1309.57 ± 2.60	1311.72 ± 0.81	1311.20 ± 0.84	-0.16	*	-0.12	ns	0.04	ns
12	1234.55 ± 1.56	1234.55 ± 1.02	1232.41 ± 1.02	0.00	ns	0.17	***	0.17	***
14	NO	1155.11 ± 1.42	1161.26 ± 2.37	-	-	-	-	-0.53	****
16	1082.12 ± 0.40	1081.34 ± 0.46	1081.97 ± 0.09	0.08	****	0.01	ns	-0.07	**
17	1055.51 ± 0.44	1053.57 ± 2.57	1055.32 ± 0.16	0.02	*	0.02	ns	-0.17	*
19	992.62 ± 0.25	978.88 ± 7.62	992.56 ± 0.25	1.40	****	0.01	ns	-1.38	****
20	966.45 ± 0.30	NO	966.53 ± 0.64	-	-	-0.01	ns	-	-

Table A7. (Cont.) The significant differences among metal treated groups for environmental *Acinetobacter* sp. (n=10)

Band areas (cm^{-1})									
Band no				**Cd vs. Pb**		**Cd vs. Ag**		**Pb vs. Ag**	
	Cd Mean ± SD	**Pb Mean ± SD**	**Ag Mean ± SD**	**% change**	***p*-value**	**% change**	***p*-value**	**% change**	***p*-value**
1	47.59 ± 5.37	60.05 ± 15.80	61.80 ± 6.29	-20.75	*	-22.99	**	-2.83	ns
6	0.16 ± 0.02	0.11 ± 0.02	0.26 ± 0.03	45.45	****	-38.46	****	-57.69	****
12	2.52 ± 0.41	2.57 ± 0.63	1.74 ± 0.19	-1.95	ns	44.83	***	47.70	***
13	8.81 ± 1.56	4.23 ± 0.70	9.94 ± 1.67	108.27	****	-11.37	ns	-57.44	****
14	0.68 ± 0.12	0.02 ± 0.01	0.44 ± 0.07	3300.00	****	54.55	****	-95.45	****
15	NO	2.22 ± 1.58	0.43 ± 0.07	-	-	-	-	416.28	**
16	2.70 ± 0.48	1.78 ± 0.96	2.56 ± 0.31	51.69	**	5.47	ns	-30.47	*
20	1.26 ± 0.31	5.83 ± 1.45	1.35 ± 0.35	-78.39	****	-6.67	ns	331.85	****
21	0.63 ± 0.17	NO	0.71 ± 0.21	-	-	-11.27	ns	-	-

Bandwidth values (cm^{-1})									
Band no				**Cd vs. Pb**		**Cd vs. Ag**		**Pb vs. Ag**	
	Cd Mean ± SD	**Pb Mean ± SD**	**Ag Mean ± SD**	**% change**	**p-value**	**% change**	**p-value**	**% change**	**p-value**
7	40.31 ± 0.91	44.71 ± 1.78	39.71 ± 0.71	-9.84	****	1.51	ns	12.59	****
8	37.64 ± 0.86	38.85 ± 0.68	36.63 ± 1.06	-3.11	**	2.76	*	6.06	****

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, non-specific; NO, not observed
The "-" indicates increases and the "+" shows decreases

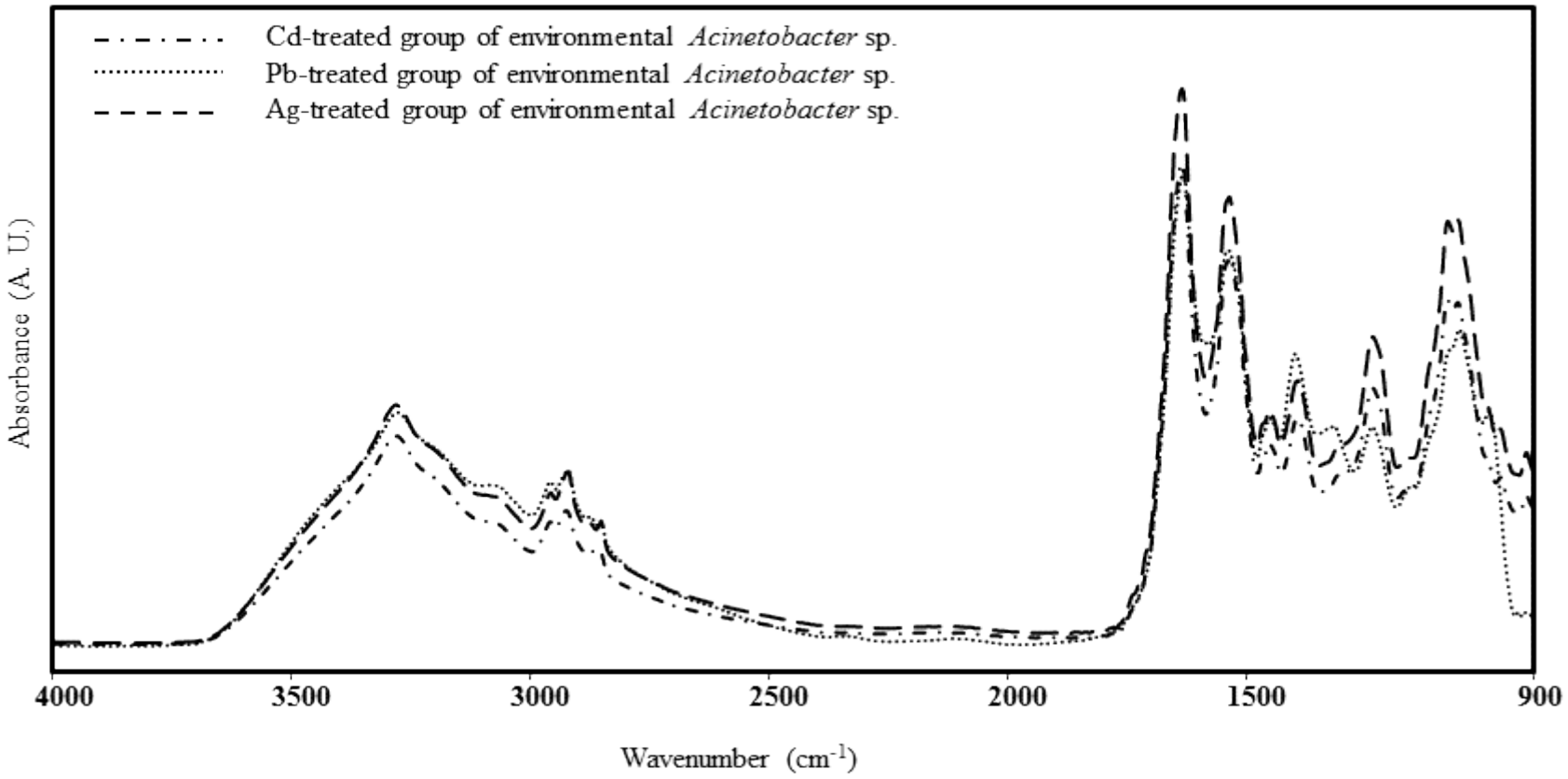

Figure A7. The average spectra of the heavy metal-treated groups of environmental *Acinetobacter* strain in the 4000-900 cm^{-1} region.

Table A8. The significant differences among metal treated groups for *A. haemolyticus* ATCC 19002 (n=10)

Band no	Frequency values (cm^{-1})								
				Cd vs. Pb		Cd vs. Ag		Pb vs. Ag	
	Cd Mean ± SD	Pb Mean ± SD	Ag Mean ± SD	% change	*p*-value	% change	*p*-value	% change	*p*-value
1	3264.55 ± 1.86	3263.70 ± 3.22	3271.95 ± 3.15	0.03	ns	-0.23	****	-0.25	****
4	2874.18 ± 0.50	2874.73 ± 0.47	2874.84 ± 0.66	-0.02	ns	-0.02	*	0.00	ns
7	1639.64 ± 0.53	1636.97 ± 0.29	1639.21 ± 0.87	0.16	****	0.03	ns	-0.14	****
8	1535.28 ± 0.91	1536.62 ± 0.97	1538.17 ± 1.18	-0.09	ns	-0.19	***	-0.10	ns
10	1394.89 ± 0.97	1391.95 ± 0.62	1393.15 ± 0.94	0.21	****	0.12	***	-0.09	*
11	1302.97 ± 1.55	1312.16 ± 1.83	1301.74 ± 3.09	-0.70	****	0.09	ns	0.80	****
12	1232.23 ± 0.65	1231.96 ± 0.52	1233.93 ± 0.96	0.02	ns	-0.14	**	-0.16	***
13	1173.42 ± 0.10	1173.84 ± 0.13	1174.80 ± 1.00	-0.04	ns	-0.12	***	-0.08	*
14	1156.98 ± 0.31	1156.14 ± 0.26	1157.14 ± 0.28	0.07	**	-0.01	ns	-0.09	***
15	1115.31 ± 0.58	1110.55 ± 1.54	1113.21 ± 0.46	0.43	****	0.19	***	-0.24	****
16	1082.19 ± 0.31	1081.60 ± 0.31	1082.03 ± 0.29	0.05	***	0.01	ns	-0.04	*
17	1056.32 ± 0.20	1053.62 ± 0.42	1056.18 ± 0.37	0.26	****	0.01	ns	-0.24	****
19	992.14 ± 0.12	991.04 ± 0.26	992.02 ± 0.71	0.11	****	0.01	ns	-0.10	****
20	965.52 ± 0.23	968.01 ± 1.43	965.94 ± 0.27	-0.26	****	-0.04	ns	0.21	****

Table A8. (Cont.) The significant differences among metal treated groups for *A. haemolyticus* ATCC 19002 (n=10)

Band no	Band area values (cm^{-1})								
				Cd vs. Pb		Cd vs. Ag		Pb vs. Ag	
	Cd Mean ± SD	Pb Mean ± SD	Ag Mean ± SD	% change	*p*-value	% change	*p*-value	% change	*p*-value
1	46.31 ± 2.97	74.50 ± 3.95	63.33 ± 7.25	-37.84	****	-26.88	****	17.64	***
2	2.51 ± 0.22	2.79 ± 0.07	2.24 ± 0.16	-10.04	**	12.05	*	24.55	****
3	5.90 ± 0.50	6.71 ± 0.22	5.68 ± 0.45	-12.07	***	3.87	ns	18.13	****
4	1.42 ± 0.12	1.61 ± 0.06	1.18 ± 0.09	-11.80	**	20.34	****	36.44	****
5	1.37 ± 0.11	1.68 ± 0.07	1.26 ± 0.12	-18.45	****	8.73	ns	33.33	****
6	0.18 ± 0.02	0.23 ± 0.03	0.27 ± 0.07	-21.74	ns	-33.33	***	-14.81	ns
8	21.48 ± 1.83	23.61 ± 1.13	19.15 ± 1.97	-9.02	ns	12.17	ns	23.29	***
9	5.32 ± 0.47	5.81 ± 0.38	4.56 ± 0.49	-8.43	ns	16.67	*	27.41	***
10	8.61 ± 0.77	9.16 ± 0.63	7.22 ± 0.79	-6.00	ns	19.25	**	26.87	***
11	4.09 ± 0.37	4.67 ± 0.50	0.64 ± 0.07	-12.42	**	539.06	****	629.69	****
12	8.05 ± 0.83	5.29 ± 0.65	7.39 ± 0.83	52.17	****	8.93	ns	-28.42	***
13	1.11 ± 0.10	2.15 ± 0.18	0.98 ± 0.14	-48.37	****	13.27	ns	119.39	****
14	0.81 ± 0.07	2.10 ± 0.18	1.00 ± 0.12	-61.43	****	-19.00	*	110.00	****
15	3.15 ± 0.31	4.18 ± 0.26	3.01 ± 0.34	-24.64	****	4.65	ns	38.87	****
16	4.30 ± 0.49	7.17 ± 0.40	3.93 ± 0.47	-40.03	****	9.41	ns	82.44	****
17	3.14 ± 0.38	4.76 ± 0.23	2.45 ± 0.33	-34.03	****	28.16	**	94.29	****
18	3.69 ± 0.44	4.51 ± 0.23	2.41 ± 0.35	-18.18	**	53.11	****	87.14	****
19	1.15 ± 0.15	2.11 ± 0.28	0.58 ± 0.10	-45.50	****	98.28	****	263.79	****
20	1.45 ± 0.18	2.45 ± 0.48	1.15 ± 0.19	-40.82	****	26.09	ns	113.04	****

Table A8. (Cont.) The significant differences among metal treated groups for *A. haemolyticus* ATCC 19002 (n=10)

Band no	Bandwidth values (cm^{-1})								
				Cd vs. Pb		Cd vs. Ag		Pb vs. Ag	
	Cd Mean ± SD	Pb Mean ± SD	Ag Mean ± SD	% change	*p*-value	% change	*p*-value	% change	*p*-value
3	18.99 ± 0.58	20.82 ± 0.65	18.72 ± 2.38	-8.79	ns	1.44	ns	11.22	*
5	4.97 ± 0.22	5.34 ± 0.27	4.82 ± 0.46	-6.93	ns	3.11	ns	10.79	**
8	37.45 ± 0.55	35.68 ± 1.61	35.69 ± 1.12	4.96	*	4.93	*	-0.03	ns

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, non-specific
The "-" indicates increases and the "+" shows decreases

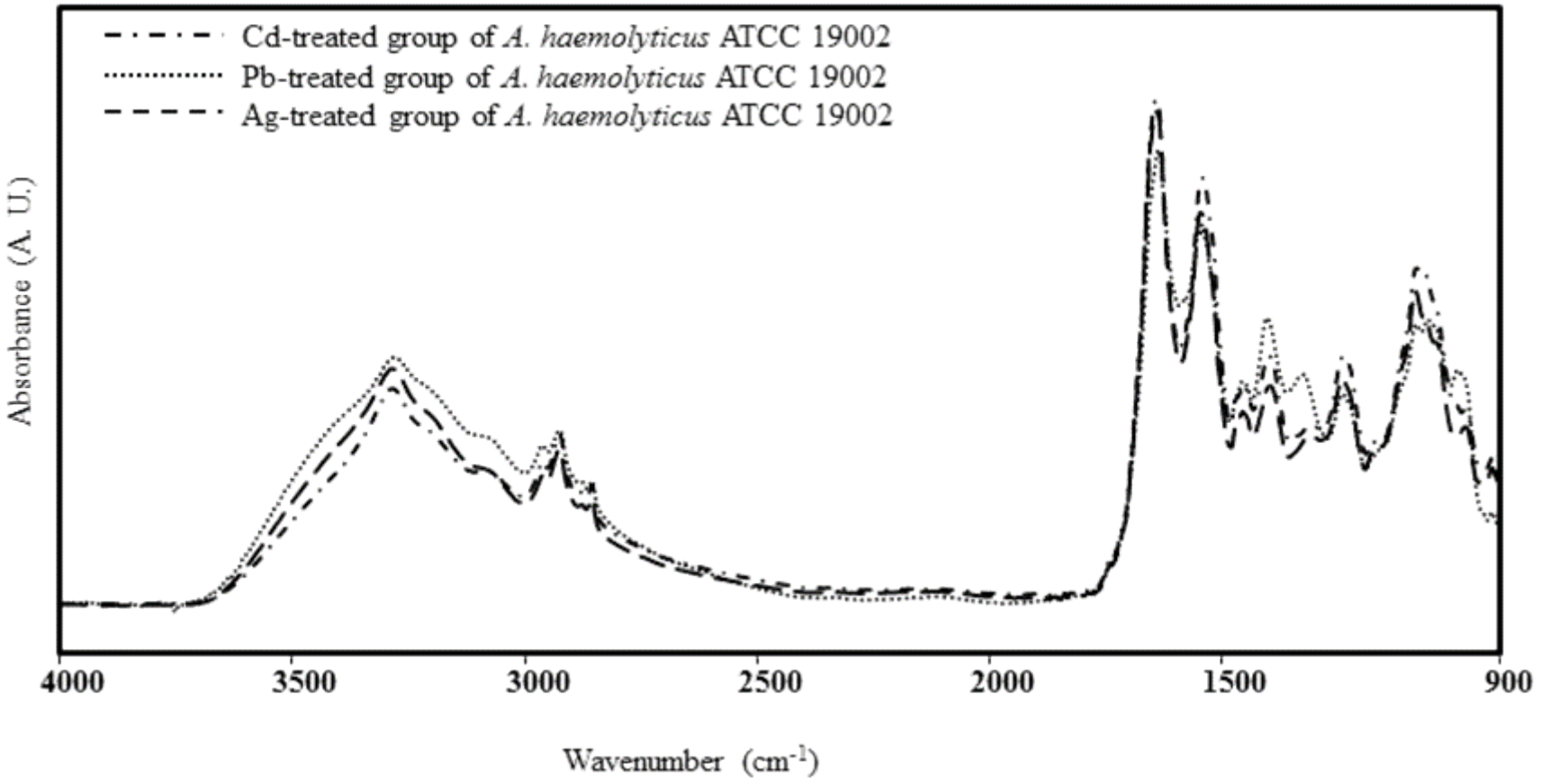

Figure A8. The average spectra of the heavy metal-treated groups of *A. haemolyticus* ATCC 19002 strain in the 4000-900 cm^{-1} region.

Table A9. The band areas with significant differences between control and heavy metal treated environmental *Acinetobacter* sp.

Band areas (cm^{-1}) for environmental *Acinetobacter* sp. (n=10)										
Band no	**Control vs. 7.8 µg/ml Cadmium**				**Control vs. 600 µg/ml Lead**			**Control vs. 15.63 µg/ml Silver**		
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**	**Pb Mean ± SD**	**% change**	***p*-value**	**Ag Mean ± SD**	**% change**	***p*-value**
1	50.04 ± 2.72	47.59 ± 5.37	5.15	ns	60.05 ± 15.80	-16.67	ns	61.80 ± 6.29	-19.03	*
6	0.30 ± 0.02	0.16 ± 0.02	87.50	****	0.11 ± 0.02	172.73	****	0.26 ± 0.03	15.38	**
7	30.01 ± 1.43	25.81 ± 3.53	16.27	ns	23.30 ± 5.68	28.80	**	26.30 ± 2.88	14.11	ns
9	3.96 ± 0.26	4.74 ± 0.74	-16.46	ns	4.51 ± 1.14	-12.20	ns	4.86 ± 0.49	-18.52	*
11	2.30 ± 0.16	2.52 ± 0.41	-8.73	ns	2.57 ± 0.63	-10.51	ns	1.74 ± 0.19	32.18	*
12	11.20 ± 0.90	8.81 ± 1.56	27.13	***	4.23 ± 0.70	164.78	****	9.94 ± 1.67	12.68	ns
13	1.47 ± 0.16	0.68 ± 0.12	116.18	****	0.02 ± 0.01	7250.00	****	0.44 ± 0.07	234.09	****
14	NO	NO	-	-	2.22 ± 1.58	-	-	0.43 ± 0.07	-	-
15	3.11 ± 0.16	2.70 ± 0.48	15.19	ns	1.78 ± 0.96	74.72	****	2.56 ± 0.31	21.48	ns
19	1.02 ± 0.09	1.26 ± 0.31	-19.05	ns	5.83 ± 1.45	-82.50	****	1.35 ± 0.35	-24.44	ns
20	0.64 ± 0.07	0.63 ± 0.17	1.59	ns	-	-	-	0.71 ± 0.21	-9.86	ns

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, non-specific; NO, not observed
The "-" indicates increases and the "+" shows decreases when compared to control group values

Table A10. The band areas with significant differences between control and heavy metal treated *A. haemolyticus* ATCC 19002

Band area values (cm^{-1}) for *A. haemolyticus* ATCC 19002 (n=10)										
Band no	**Control vs. 80 µg/ml Cadmium**				**Control vs. 900 µg/ml Lead**			**Control vs. 15.63 µg/ml Silver**		
	Ctrl Mean ± SD	**Cd Mean ± SD**	**% change**	***p*-value**	**Pb Mean ± SD**	**% change**	***p*-value**	**Ag Mean ± SD**	**% change**	***p*-value**
1	56.25 ± 7.51	46.31 ± 2.97	21.46	**	74.50 ± 3.95	-24.50	****	63.33 ± 7.25	-11.18	*
2	2.41 ± 0.23	2.51 ± 0.22	-3.98	ns	2.79 ± 0.07	-13.62	***	2.24 ± 0.16	7.59	ns
3	5.89 ± 0.48	5.90 ± 0.50	-0.17	ns	6.71 ± 0.22	-12.22	***	5.68 ± 0.45	3.70	ns
4	1.40 ± 0.13	1.42 ± 0.12	-1.41	ns	1.61 ± 0.06	-13.04	***	1.18 ± 0.09	18.64	***
5	1.50 ± 0.11	1.37 ± 0.11	9.49	*	1.68 ± 0.07	-10.71	**	1.26 ± 0.12	19.05	****
6	0.36 ± 0.03	0.18 ± 0.02	100.00	****	0.23 ± 0.03	56.52	****	0.27 ± 0.07	33.33	***
8	20.15 ± 3.54	21.48 ± 1.83	-6.19	ns	23.61 ± 1.13	-14.65	**	19.15 ± 1.97	5.22	ns
10	7.72 ± 1.29	8.61 ± 0.77	-10.34	ns	9.16 ± 0.63	-15.72	**	7.22 ± 0.79	6.93	ns
11	1.46 ± 0.25	4.09 ± 0.37	-64.30	****	4.67 ± 0.50	-68.74	****	0.64 ± 0.07	128.13	****
12	8.33 ± 1.54	8.05 ± 0.83	3.48	ns	5.29 ± 0.65	57.47	****	7.39 ± 0.83	12.72	ns
13	1.12 ± 0.18	1.11 ± 0.10	0.90	ns	2.15 ± 0.18	-47.91	****	0.98 ± 0.14	14.29	ns
14	0.94 ± 0.15	0.81 ± 0.07	16.05	ns	2.10 ± 0.18	-55.24	****	1.00 ± 0.12	-6.00	ns
15	3.42 ± 0.54	3.15 ± 0.31	8.57	ns	4.18 ± 0.26	-18.18	***	3.01 ± 0.34	13.62	ns
16	4.60 ± 0.83	4.30 ± 0.49	6.98	ns	7.17 ± 0.40	-35.84	****	3.93 ± 0.47	17.05	ns
17	3.08 ± 0.61	3.14 ± 0.38	-1.91	ns	4.76 ± 0.23	-35.29	****	2.45 ± 0.33	25.71	**
18	3.53 ± 0.78	3.69 ± 0.44	-4.34	ns	4.51 ± 0.23	-21.73	***	2.41 ± 0.35	46.47	****
19	0.91 ± 0.19	1.15 ± 0.15	-20.87	*	2.11 ± 0.28	-56.87	****	0.58 ± 0.10	56.90	**
20	1.35 ± 0.33	1.45 ± 0.18	-6.90	ns	2.45 ± 0.48	-44.90	****	1.15 ± 0.19	17.39	ns

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, ****$p < 0.0001$; ns, non-specific

The "-" indicates increases and the "+" shows decreases when compared to control group values

CURRICULUM VITAE

Personal Information

Surname, Name	:	Özaktaş, Tuğba
Nationality	:	Turkish
Date and Place of Birth	:	20 February 1979, Ankara
Marital Status	:	Single
Phone	:	+90 312 210 64 76
E-mail	:	tugbaozaktas@gmail.com
Foreign Languages	:	English

Education Degrees

February, 2015	:	**Ph.D.**, Biology Dept., METU
December, 2007	:	**M.Sc.**, Biology Dept., METU
June, 2004	:	English Preparatory Program, METU
September, 2003	:	**M.Sc.**, Educational Sciences, Ankara University
June, 2002	:	**B.Sc.**, Biology Dept. - Branch of Molecular Biology and Biotechnology, Ankara University

Work Experience

2005-	:	Research Assistant at Biological Sciences, METU

Publications

A) Articles

Ozaktas, T., Taskin, B., Gozen A. G. (2012). High level multiple antibiotic resistance among fish surface associated bacterial populations in non-aquaculture freshwater environment. Water Research, 46 (19), 6382-6390.

B) Presentations in International Meetings

Ozaktas, T., Gozen, A. G., Severcan, F. (2014). The Comparison of Macromolecular changes in Different *Acinetobacter* Isolates upon Neomycin Exposure. FEBS EMBO, Paris- France (Poster Presentation).

Ozaktas, T., Taskin, B., Gozen, A. G. (2009). Isolation and Identification of Multiple Antibiotic Resistant Bacteria from Surface Mucus of Freshwater Fish. 3rd Congress of European Microbiologists (FEMS), Gothenburg-Sweden (Poster Presentation).

Ozaktas, T., Citir, G., Gozen, A. G. (2008). Multiple Antibiotic and Heavy Metal Resistance of Surface Mucus Associated Bacterial Populations on Freshwater Fish from Lake Mogan. XII. International Congress of Bacteriology and Applied Microbiolgy (IUMS), Istanbul- Turkey (Poster Presentation).

C**) Presentations in National Meetings**

Ozaktas, T., Gozen, A. G., Severcan, F. (2014). Çevresel Kaynaklı *Acinetobacter* İzolatında Ampisilin veya Kadmiyum Varlığında Meydana Gelen Makromoleküler Değişiklikler. 8. Ulusal Moleküler ve Tanısal Mikrobiyoloji Kongresi, Ankara-Türkiye (Poster Presentation).

Ozaktas, T., Gozen, A. G., Severcan, F. (2014). Farklı Antibiyotik Konsantrasyonlarının Çevresel Bakteri İzolatında Oluşturduğu Makromoleküler Değişimlerin ATR-FTİR Spektroskopisi ile Belirlenmesi. 22. Ulusal Biyoloji Kongresi (UBK), Eskişehir- Türkiye (Poster Presentation).

Ozaktas, T., Gozen, A. G. (2012). Tatlı Su Balığının Yüzey Mukusu Bakteri Populasyonunun Mevsimsel Olarak İncelenmesi. Fisheries and Aquatic Sciences– Balıkçılık ve Akuatik Bilimler (FABA) 2012 Sempozyumu, Eskişehir- Türkiye (Poster Presentation)

Ozaktas, T., Gozen, A. G. (2010). Quantitative Effect of Seasons on the Freshwater Fish Surface Mucus Associated Bacterial Populations. XX. Ulusal Biyoloji Kongresi (UBK), Denizli- Türkiye (Poster Presentation).

Ozaktas, T., Gozen, A. G. (2007). Gümüş Balığının Yüzey Mukusundan İzole edilen Bakterilerdeki Yüksek Çoklu Antibiyotik Direnci. XV. Ulusal Biyoteknoloji Kongresi, Antalya- Türkiye (Poster Presentation).

Participations in International Symposiums:

The 2nd Annual Horizons in Molecular Biology and Genetics Symposium, 2009, Bilkent University, Ankara-Turkey.

Biotech-METU 2009, International Symposium on Biotechnology: Developments and Trends, Middle East Technical University, Ankara-Turkey.