# HEAVY METAL SEQUESTRATION
# BY A REPRESENTATIVE GROUP OF BIOLOGICAL ORGANISMS:
# A STUDY IN THEIR COMPARTMENTALIZATION

**By**

**Jacob Joseph Goldberg**

A dissertation submitted to the Graduate Faculty in Biology in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York

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# ABSTRACT

HEAVY METAL SEQUESTRATION BY A REPRESENTATIVE GROUP OF BIOLOGICAL ORGANISMS:

A STUDY IN THEIR COMPARTMENTALIZATION

By

Jacob Joseph Goldberg

Advisor: Professor Thomas E. Jensen

The ability of cells to take up metal and to bioconcentrate them in different inclusions/organelles including their capsule, cell wall, cytoplasm, and polyphosphate bodies was tested. Nine prokaryotic species and two eukaryotic species were used. Among the prokaryotic type of species were

cyanobacteria, gram-positive and gram-negative bacteria, and yeast. The cyanobacteria were *Plectonema boryanum*, *Synechococcus leopoliensis* and *Gloeocapsa alpicola,* three-gram positive bacteria *Bacillus subtilus*, *Staphylococcus aureus*, and *Arthrobacter globiformis*, three-gram negative bacteria, *Acinetobacter calcoaceticus*, *Pseudomonas aeruginosa,* and *Escherichia coli*, two eukaryotic species, *Saccharomyces cerevisiae,* and *Rhodotorula rubra*. All organisms contain polyphosphate bodies under the right conditions. The cyanobacteria were grown in Modified Fitzgerald's Media and, the gram positive and gram negative bacteria were grown in nutrient broth and the yeast cells were grown in Difco-Bacto Sabouraud broth. The cells were exposed to seven different metals, Al, Cd, Cu, Mn, Ni, Pb, and Zn. Exposure was done at the same concentrations of 20ppm for two hours, then the cells were embedded in Epon according to Luft's procedure The cells were analyzed using the STEM mode of a transmission electron microscope in conjunction with a PGT IMIX EDX. The results were then analyzed using quantitative analysis program. Analysis was done on the capsule, cell wall, cytoplasm, and polyphosphate bodies. Approximately 20 cells were analyzed for each heavy metal exposure. The averages of the elements present were calculated. Analysis of the four different parts of the cell revealed that in cells exposed to all the metals with an exception of Cd,

the metal will bioconcentrate mainly in the PPBs. The data converted into real numbers using the shape of the organisms and one of these formulas for a cylindrically shaped organism “pi x radius$^2$ x height”, for spherically shaped the formula “(4/3) x pi x radius$^3$”. Giving us μ/cm$^3$ these data indicate that different cell components in the test species sequester metals differentially, and the different metals are also sequestered in the different cell components differentially.

This Work is dedicated to my wife AVIVA, who helped me with the preparation of this work and my mother JUDITH and to my children YITZCHAK, CHANA, CHAIM, AND CHAYA, who are fortunate to live in the free, blessed, democratic, United States of America.

In Memory of my father, mentor and friend Izaak who did not live to see this moment. And to my best friend Ira Silverstein who was taken from us at too young an age and did not live to celebrate this completion with me. And to all of my family members who were murdered by the Nazis.

May their souls enjoy eternal life with all the other righteous men and women. Conceal them in the mystery of thy wings forever. May the Lord be their inheritance and may they repose in peace

Grandparents:

Jacob Joseph
Chana Rachel

Uncles:

Abraham
Mendel
Moses

Aunt:
Raizel (Shoshana)

**The Purely Righteous**

Do not complain about wickedness
They increase, righteousness;

Do not complain about heresy

They increase faith;

Do not complain about ignorance
They increase wisdom.

Rabbi Avraham Yitzchak HaCohen Kook

# ACKNOWLEDGMENTS

As I draw to the end of the writing of my "magnum opus" I feel that there are a few people that I must acknowledge, and thank. In my life I have had five mentors. Each one has mentored me either in spirit or in knowledge and at times in both. My first mentor was my father, Izaak Goldberg M.D., a physician, an historian, and at times a philosopher. All of his values he instilled in me. He was a survivor of the Holocaust, and escaped the concentration camp, called Auschwitz, he saw the infamous Dr. Mengele with his own eyes, lost his home and whole family. He came to the United States his new home and with nothing, started a thriving medical practice in the Bronx, not far from Lehman College, where I completed my studies. He was known to have many intelligent sayings and idioms, but two that carried him though his life adopted from the Talmud Brachot 10a, were "Even if a sharp sword is resting on your neck, never give up hope" and his second idiom was one he adopted from Ralph Waldo Emerson, "Every man I meet is in some way my superior." My father taught me many things but most of all how to be a father and a friend to my children.

My second mentor was Rabbi Chaim Druckman, my religious teacher, my *Rebbi*, and my friend. Rabbi Druckman was a member of the Israeli

parliament “The Knesset” and is the head of *Yeshviat Or Etzion*, the Yeshiva that I spent most of my late teens and early twenty’s studying in. He taught me to always question, keep an open mind, and think for yourself, and never to follow blindly. Do not be robots; use the skills G-D gave you. He taught me, to be kind to your students, open their minds, and their hearts to knowledge, respect them, and always make time for their questions and their comments, even if that time was at 12:00 or 1:00 AM.

The next mentors are a father and his son, two people that I never met; yet they have shaped my entire being profoundly as they have shaped the lives of many others. These two are the First Chief Rabbi of the Land of Israel, Rabbi Abraham Izaak HaCohen Kook. From him I learned tolerance even in the face of adversity, humility, and how to show kindness to others of all faiths and social status. He was a true angel among men.

His only son Rabbi Tzvi Yehudah HaCohen Kook. The head of the Mercaz Harav Kook Talmudical Academy in Jerusalem Israel. An institution established by his father and later named for his father. A place where I sat and learned , *Hashem’s* Torah. The mentor of my mentor, a man whom I never met, yet feel very close to A man I learned much from, including

honor, how to conduct oneself with dignity and a man I learned much philosophy from and a man I have adopted an idiom from. *Btzipiah Legeulah Hashlema*, we are waiting for the final redemption.

My last mentor and most current one is Dr. T.E. Jensen, a man who made a place for me in his lab. A man who trusted me to teach his courses, and who trusted me and allowed me to mentor undergraduates, a man who directed me in my research and had enough confidence in me to allow me to take on a project so dear to him. I thank Dr. Jensen for teaching me all the methods I know in electron microscopy but for also allowing me to work at my own pace and supporting me throughout my work. I also owe Dr. Jensen a debt of gratitude for allowing me to go to so many conferences and see this wonderful and beautiful country.

I thank Mr. Michael Baxter for helping me with his advice and his prodding and most importantly his friendship. It was always nice to walk into the lab and have a conversation with Mike, whether it was about current events, religion, or that occasional conversation about science.

A special thanks goes out to Dr. William Corpe, who at the beginning of my career at Lehman College-CUNY was there every morning bright and early

with a kind word and a piece of advice, or a story from his time as a Marine in World War II. It was nice to have been able to spend some time with him before his retirement.

Thanks to Hugo Gonzalez who was the first to teach me how to use the IMIX and then became a student of mine. Thanks to Gustavo Nuñez for all his help, best wishes to you and yours. And To my good friend Rajendra Gharbaran for all his help.

I would like to thank Dr. Pattan Dial of Coral Springs, Florida, who was the only one willing and able to help me complete the statistics, for my "opus magnum". And who took time out from his busy schedule to meet with me for hours until I knew and understood what I was doing.

I want to thank my wife Aviva for putting up with all my tirades, my wonderful and beautiful children, my oldest son Yitzchak who took an interest in my work at a very early age may he grow up to be a *talmid chochom* a *yireh shomayim* and most importantly a good person may the light of G-D shine on his face always. To my oldest daughter Chana, who always made me appreciate life even if it was through her eyes may *Hashem*

bless her with wisdom, strength, piety and the ways of our Holy Torah, grow up to be an *Aishes Chayil* a women of Valor learn from your name sake, your great grand mother how to have a family and a career. To my younger son Chaim the straight man in a comedy routine, who can keep me entertained with his tall tales for hours on end, I hope you are able to maintain your good personality and character forever. May *Hashem* grant him the wisdom to learn the secrets and the pearls of the Torah, the strength to fulfill all of the commandments therein and may he be a source of light to his people and all of the nations in the world. And then there is Chaya (Shania), still just two years old but a bundle of joy and laughs. Always there with her older brother Chaim to greet me when I come home at night. May *Hashem* give her the intelligence to study His Holy Torah, the wisdom and the sense to live it. May Hashem give her the ability to embody the meaning of her names Chaya, “Life” may she with, G-D’s help, live life to the fullest and her English name Shania which is actually Ojibwa for "I'm on my way." Watch out world here she comes. To all my children may I bless you that you find favor and intelligence in the eyes of G-D and Man. Remember the words of your great great-grand father King Solomon “listen my son to the *mussar* of your father, do not forsake the Torah of your Mother”. May you all grow up in the way of our Torah find a love for *Eretz*

*Yisrael* and *Am Yisrael* and follow in the ways of Rav Kook and his disciples.

Rudyard Kipling wrote a poem to his son entitled “If”, children these words are good words to live by:

If you can keep your head when all about you
Are losing theirs and blaming it on you;
If you can trust yourself when all men doubt you,
But make allowance for their doubting too:
If you can wait and not be tired by waiting,
Or, being lied about, don't deal in lies,
Or being hated don't give way to hating,
And yet don't look too good, nor talk too wise;
If you can dream -- and not make dreams your master;
If you can think -- and not make thoughts your aim,
If you can meet with Triumph and Disaster
And treat those two impostors just the same:
If you can bear to hear the truth you've spoken
Twisted by knaves to make a trap for fools,
Or watch the things you gave your life to, broken,

And stoop and build 'em up with worn-out tools;

If you can make one heap of all your winnings

And risk it on one turn of pitch-and-toss,

And lose, and start again at your beginnings,

And never breathe a word about your loss:

If you can force your heart and nerve and sinew

To serve your turn long after they are gone,

And so hold on when there is nothing in you

Except the Will which says to them: "Hold on!"

If you can talk with crowds and keep your virtue,

Or walk with Kings -- nor lose the common touch,

If neither foes nor loving friends can hurt you,

If all men count with you, but none too much:

If you can fill the unforgiving minute

With sixty seconds' worth of distance run,

Yours is the Earth and everything that's in it,

And -- which is more -- you'll be a Man, my son!

-- Rudyard Kipling

I would like to thank my mother, Judith Goldberg may she live *ad meoh v'esrim* that supported me and helped Aviva and me when ever possible.

I have taught at Lehman for ten years, during that time I have had over two thousand students some I have gotten to know and some unfortunately not. But I have learned much from them maybe even more than I was able to teach them, to take a line from the Ethics of our Fathers a book in the Holy books of the *Mishnayos*, "I can learn something from each and every person."

And last but not least I must thank G-D for all his help, through these years. I did my work with very little help from others I made my own solutions grew my own cultures cut my own sections and generally did everything on my own, with G-D's help. Fore that is truly the only way to learn.

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## INTRODUCTION

The science of quantitative x-ray microanalysis has been available to researchers for several decades. Yet due to the problems inherent in dealing with the non-uniform nature of biological specimens, quantitative x-ray microanalysis has been more or less limited to non-biological specimens, like metals, geological samples, etc. Beginning in the late 1960s through the mid 1980s, quantitative analysis took a great leap forward, with the work done by Theodore Alvin Hall and his colleagues. This work has resulted in the Hall continuum method for biological quantitative x-ray microanalysis.

During the 1980s to the present, Heldal and his associates (Heldal 1985 and 1993) developed a method for calculating the actual mass of biological specimens using the information from quantitative analysis and qualitative biological microanalysis.

When a metal is sequestered in a cell there is disagreement in regard to what cell part or component is most important in the binding. In some studies the

capsular material is indicated, in other studies the cell wall, in still others the cytoplasm, and lastly cell inclusions called polyphosphate bodies (PPBs). We now have the instrumentation and methodology to solve this problem.

## LITERATURE REVIEW

Microbes have evolved a number of ways to tolerate environments, which are rich in heavy metals. These abilities have allowed them to survive while organisms that lack these adaptations cannot flourish in these niches. Information from pure culture and consortia studies suggests technologies that may enable us to deal with problems caused by heavy metals, especially in water, that we desperately need to conserve. Intracellular metal sequestering has been reported in a number of studies, involving different organisms.

Numerous investigators have carried out the study of metal uptake that occurs in microbes. It appears at this time that whichever metal is exposed to any organism will be sequestered, if the organism does not have an exclusion mechanism. Many microbes possess plasmids which confer on them resistance to a heavy metal. This study does not investigate this interesting phenomenon.

In this study I tested the hypothesis that PPBs are the main sequestering sites for most heavy metals in many microbes. Overall the data will have application in a number of biological areas including health. Heavy metals in water have been shown to be a problem in regard to human health. Some are known carcinogens while others most likely do cell damage to specific cell inclusions as well as interfering with the normal biochemical reactions in cells.

The exact place that heavy metals are sequestered in microbial cells has been controversial. The metal may sequester mainly in the capsule (sheath), cell wall, cytoplasm or a cell inclusion such as the PPBs. The sequestering also may occur in more than one site, with the bulk of the metal still being sequestered in a specific site. This is what is evident in this current study.

A number of studies have suggested the capsule or sheath as the main sequestering site. Strains of *Pseudomonas atlantica* were isolated as a

planktonic organism and as a periphyte from the sea at Scripps Institute of Oceanography at LaJolla (Corpe, 1970). Cultures produced a voluminous, acidic polysaccharide that formed insoluble precipitates with a variety of heavy metals including Cu, Pb, and Zn among others (Corpe, 1975). Since all strains grew readily in high concentration of copper, it was believed possible that the anionic polymer protected the cells. However, mutants lacking the voluminous polymer were equally tolerant of copper (Corpe et al., 1976). Horikoshi et al. (1979 and 1981) studied uranium accumulation in 10 Gram-negative bacteria, 13 actinomycete, 11 yeasts and 18 additional fungi. Two of the actinomycetes accumulated uranium in significant amounts: *Actinomyces levoris* and *Streptomyces viridochromogenes*. EDTA released the metal, indicating surface binding, probably capsular. Karapanagiotis et al. (1990) have shown that bacterial exopolymer and fulvic acid extracted from a sewage plant bind Cu, Cd, Ni and Zn. Dune and Bull (1983) have shown specifically that Cu binds to walls of bacteria in activated sludge. Loaec et al. (1997) extracted exopolysaccharide from *Alteromonas madeodii* and found that it bound Pb, Cd and Zn.

Cell wall sequestering of metals has been suggested by a number of studies. Beveridge (1989) discussed how the design of bacterial cell walls allows them to sequester metals.

The wall and especially the S-layers seem to be best adapted for this function. Hambuchers-Berhin and Remacle (1990) show that Cd uptake in two strains of *Alcaligenes eutrophus* is by the cell envelope and the peptidoglycan layer. Reddy and Prasad (1990) review heavy metal binding proteins. Many of these metal-binding proteins are inducible. The exopolysaccharide of *Pseudomonas* binds U with 96 mg U/mg polymer (Marques et al., 1990). In a cyanobacterium Verma and Singh (1990) found Cu binds to the wall and is then taken into the cell by a metabolism dependent system. Trevors and Cotter (1990) in a review of Cu uptake and toxicity report that this two step uptake is generally found in the microbes. However, they point out that *Pencillium ochro-chloron* Cu is taken up by reversible passive receptors. The cell walls of lake sediment bacterial isolates bind significant amounts of Se (Silverberg et al., 1976). The main bacteria in this study were *E. coli., Pseudomonas, Aeromonas,* and *Flavobacterium* species. Other bacteria cause metals to be deposited on capsular material. A number of bacteria deposit Fe and Mn into the capsule

(Ghiorse and Hirsch, 1978; Cowen and Silver, 1983). The latter authors suggest that as this phenomenon occurs, it causes the bacteria to sink. Bossrez et al. (1997) found that isolated cell walls of *Enterococcus hirae* bind Ni. Penicillin resistant cell walls from this organism bound more Ni. Binding of Pb, Cd and Cu by carboxylate and amine groups in *Aspergillus niger* suggested cell walls as the sequestering site (Kapoor and Viraraghavan, 1997). Mullen et al. (1989) in a study using 4 bacteria suggest La is bound on the surface of the cells while Ag is bound here and also in the cytoplasm.

In most of these studies, as well as those using capsular material, only that particular inclusion was tested. In almost all studies on sequestering by other cell components no data was obtained and if so in what amounts.

Intracellular sequestering of metals has also been reported in a number of studies. Jensen and his co-workers have investigated the heavy metal binding of polyphosphate bodies in laboratory cultures of microbes. They have shown that these bodies bind significant amounts of Ba, Mg, Mn, Cd,

Co, Cu, Hg, Ni, Pb, and Zn (Baxter and Jensen, 1980a; Jensen et al., 1982a; Jensen et al., 1982b; Rachlin et al., 1984; Jensen et al., 1986; Rai et al., 1990). Kunst and Roomans (1985) have shown that Cr, Ni, Cd and Pb were also sequestered in polyphosphate bodies. Sicko-Goad and Stoermer (1979) showed that Pb and Cu were sequestered in polyphosphate bodies in *Diatoma tenue* var. *elongatum*. Similar results were reported for *Plectonema* and *Schizorthirx* species exposed to Zn (Lazinski and Sicko-Goad, 1990). Lazinski and Sicko-Goad (1990) found that cells of the diatom *Cyclotella meneghinheusin* showed an increase in polyphosphate synthesized when cells were treated with chromium. Other morphological modifications were observed as well.

Anderson et al. (1992) reported $SrSO_4$ in the vacuole of a radiolarian indicating a similar sequestering. In the case of Sr the binding was in a dense body, which also contained P. Other workers have also shown polyphosphate bodies can bind heavy metals. Pettersson et al. (1988) showed that Al is sequestered in polyphosphate bodies of *Anabaena cylindrica*. They have shown that this microbe can sequester up to 3.3% of its dry weight in Al (Pettersson et al., 1985). Scott and Palmer (1990) have

shown that *Arthrobacter* and *Pseudomonas* precipitate Cd in dense bodies with high P content. These bodies are most likely polyphosphate bodies. Similar results were report on Zn deposition in *Xanthomonas maltophilis* has been made by Sakurai et al. (1990). Vymazal (1984) also reported that Zn is bioaccumulated into polyphosphate bodies. All phosphates including polyphosphates, are highly charged anions and will therefore attract strongly to cations (Peverly et al., 1978).

Sequestering a metal in the cytoplasm also seems to occur. Jensen and co-workers have shown that in cyanobacteria such as *Plectonema boryanum* a small peak is obtained for Cd, Ca, Hg, Pb and Zn when exposed to the metals singularly. These were qualitative analysis studies so no real amounts were obtained (Jensen et al., 1984).

We have seen that many studies have proved that microbial cells will take up metals. When the metal is sequestered in a cell there is disagreement in regards to what part of the cell or component is most important in the binding of a metal. We have seen that some studies claim that the capsule is

the site of sequestration, while other studies claim that it is the cell wall, still other studies claim it that the cytoplasm, and lastly some studies indicate that other sites are the polyphosphate bodies (PPBs) that are involved.

We now have the instrumentation and methodology to solve this problem. We have an energy dispersive x-ray spectrometer (EDX) as part of our transmission electron microscope (TEM). The TEM has a scanning mode and a scanning transmission mode (STEM). It is possible to locate a cell in the TEM then switch to the STEM mode.

We can then determine the quantitative and qualitative values of the elements. This data can then be extrapolated to a whole cell. And be expressed in percentages and/or actual amounts in grams or smaller units. These data can then be related to the individual sites in the cell.

Test Species

In this study I chose four types of organisms, nine prokaryotic species and two eukayotic species. Among the prokaryotic type of species were cyanobacteria, gram-positive and gram-negative bacteria. In addition I used two different strains of yeast. The cyanobacteria I have chosen are *Plectonema boryanum* (UTEX 581) (Figure 1a&b), *Synechococcus leopoliensis* (UTEX B 2434)(Figure 2*)*, and *Gloeocapsa alpicola* (UTEX B 589)(Figure 3); I chose three gram positive bacteria *Bacillus subtilus* Presque Isle collection cat. #620 (Figure 4), *Staphylococcus aureus* Presque Isle collection cat. # +4651 (Figure 5), and *Arthrobacter globiformis* Presque Isle collection cat. # 607 (Figure 6). In addition to these two groups, I chose to examine three-gram negative bacteria, *Acinetobacter calcoaceticus* Presque Isle collection cat. #346 (Figure 7), and *Pseudomonas aeruginosa,* Presque Isle collection cat. #+99 (Figure 8), and *Escherichia coli* Presque Isle collection cat. # 336 (Figure 9). To make the study an all-encompassing one I also chose two eukaryotic species (yeast), *Saccharomyces cerevisiae,*

Presque Isle collection cat. #1015 (Figure 10), and *Rhodotorula rubra* Presque Isle collection cat. # 1010 (Figure 11). All of these organisms contain polyphosphate bodies under the right conditions.

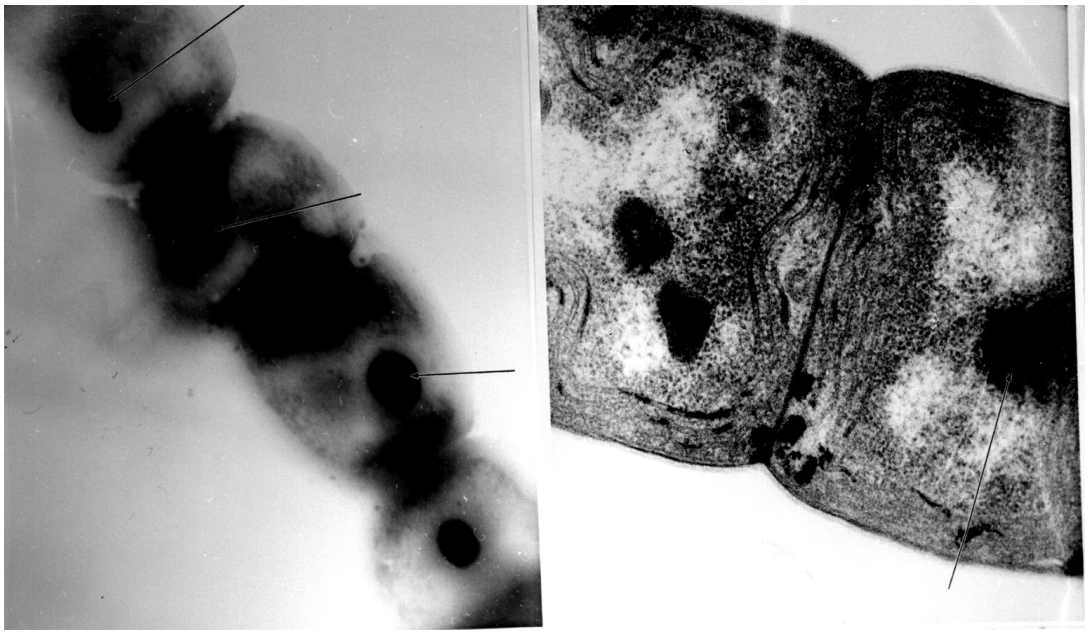


Figure 1a Photomicrograph of an air-dried *Plectonema boryanum* magnified 20,000X. Arrows point to Poly Phosphate Bodies.

Figure 1b Photomicrograph of an cell of *Plectonema boryanum* sectioned using a diamond knife, magnified 40,000X. Arrows point to Poly Phosphate Bodies.

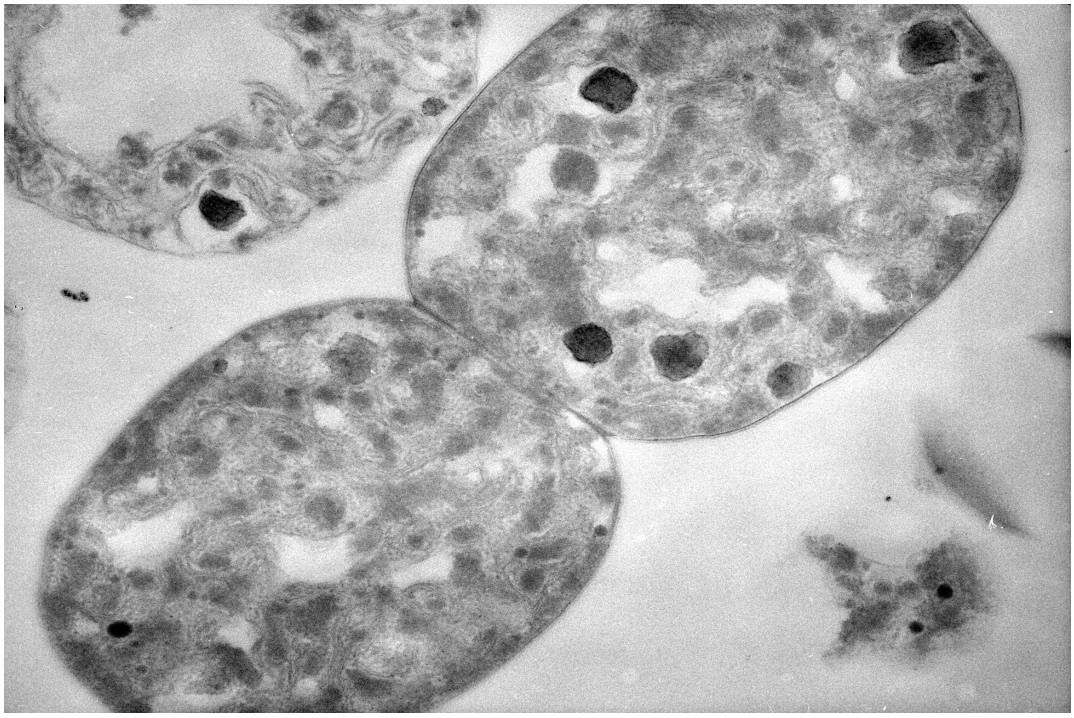


Figure 2. Thin section cell of *Synechococcus leopoliensis*. magnification is 40000X.Electron dense areas are PPB's.

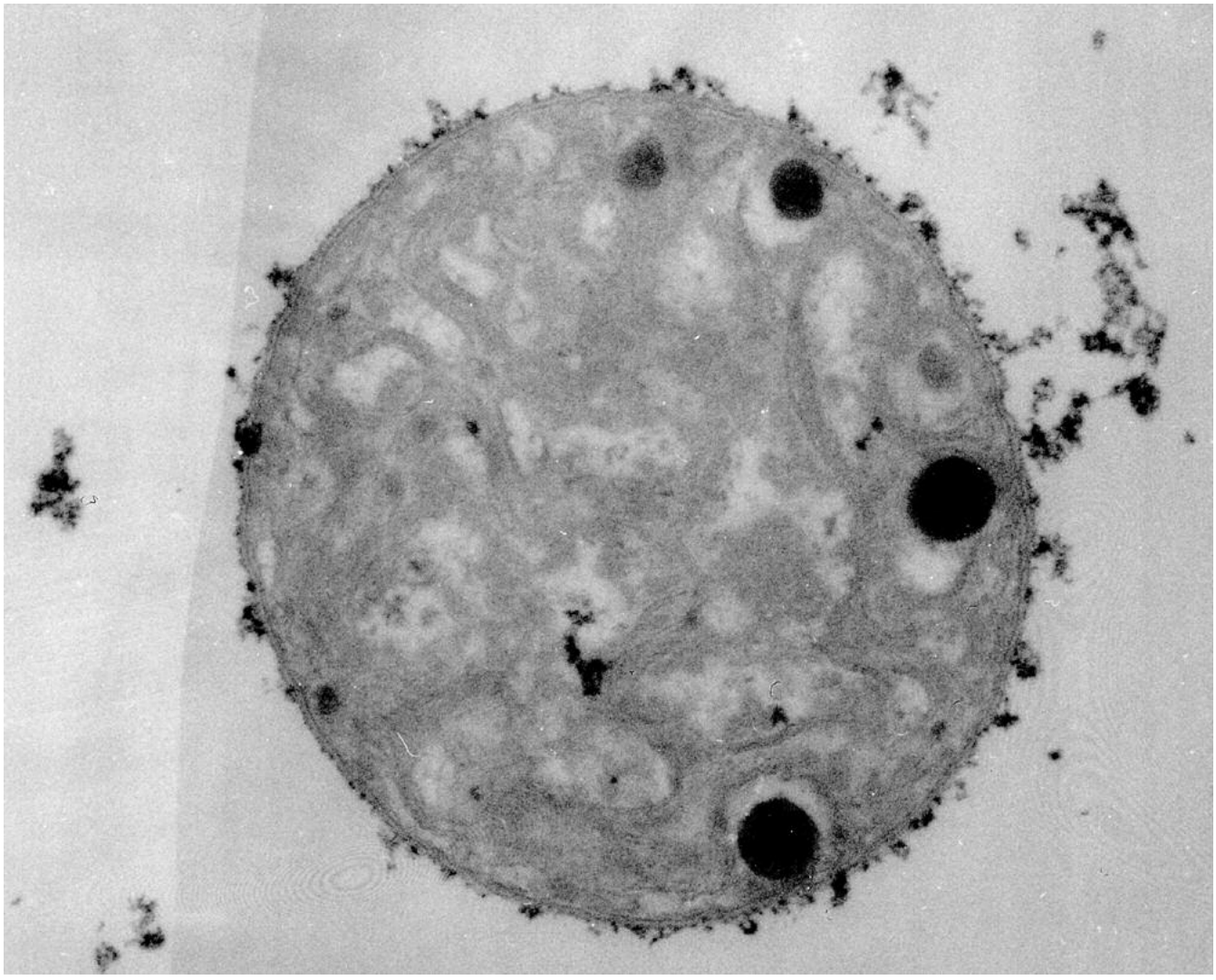


Figure 3. Thin section cell of *Synechococcus leopoliensis*. magnification is 40000X.Electron dense areas are PPB's.

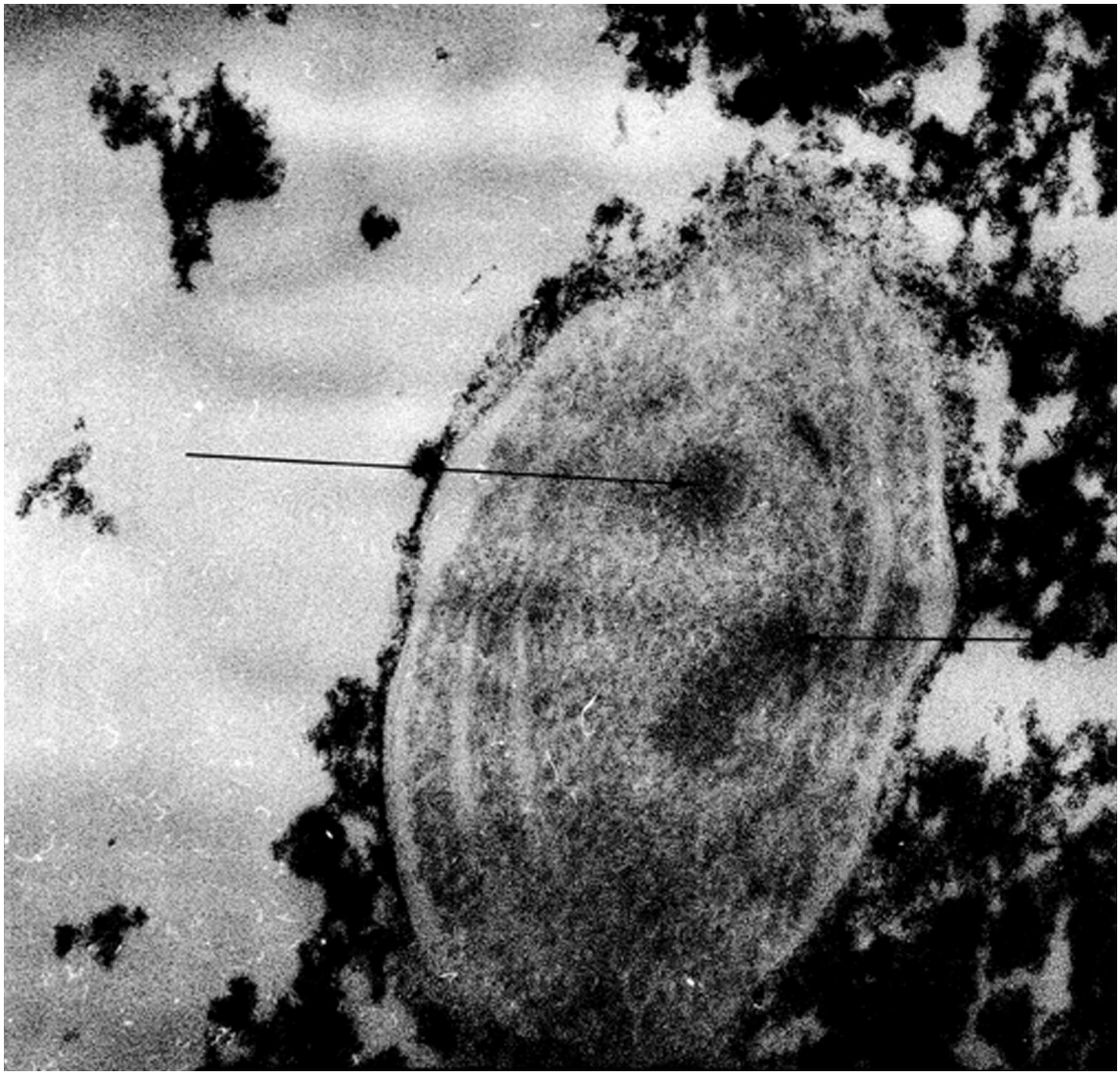


Figure 4. Thin section of *Bacillus subtilus,* Electron dense bodies are PPB's magnified 30,000X. Arrows point to PPBs.

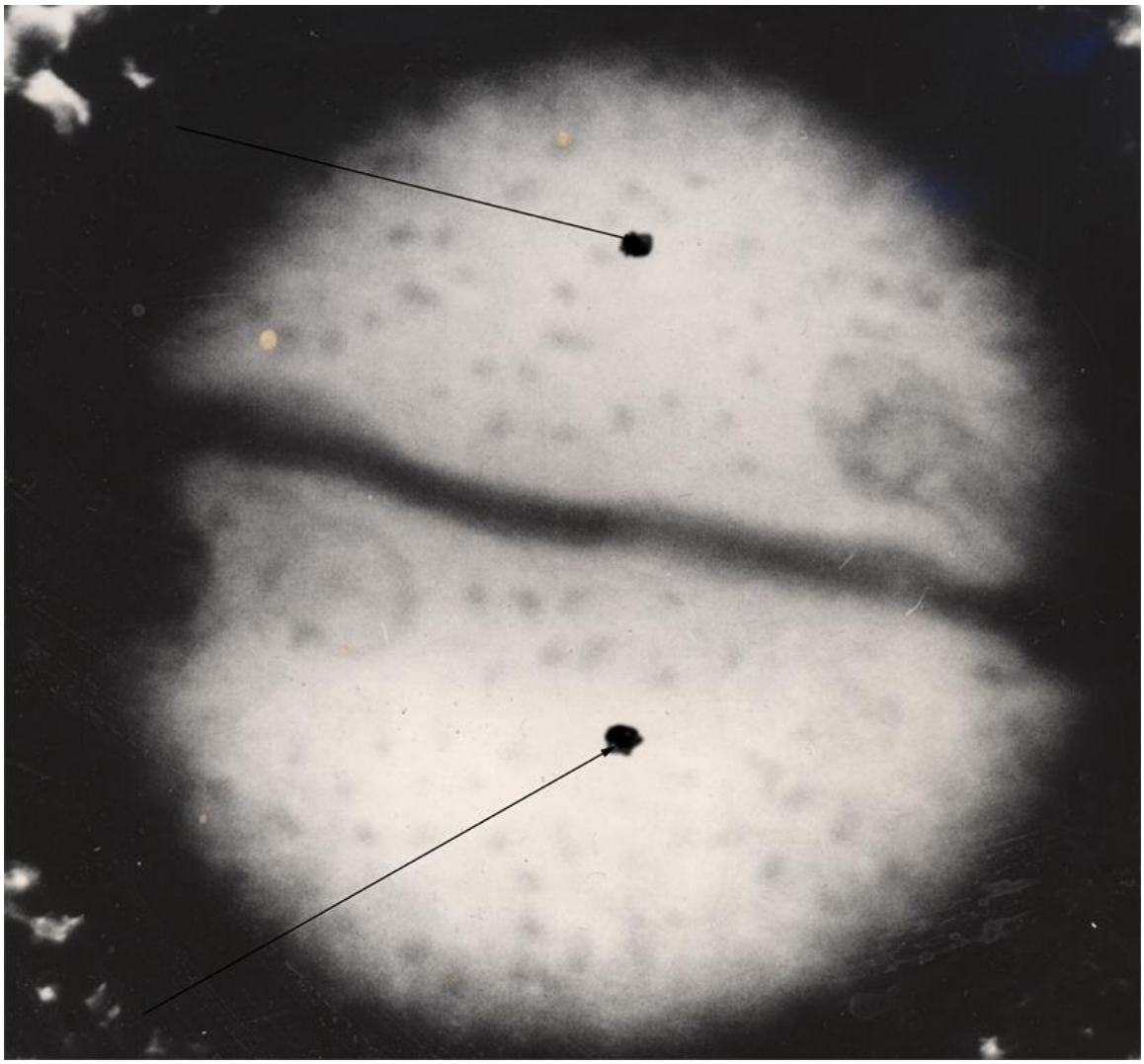


Figure 5. Air dried cell of *Staphylococcus aureus,* Electron dense bodies are PPBs magnified 30,000X. Arrow points to PPBs.

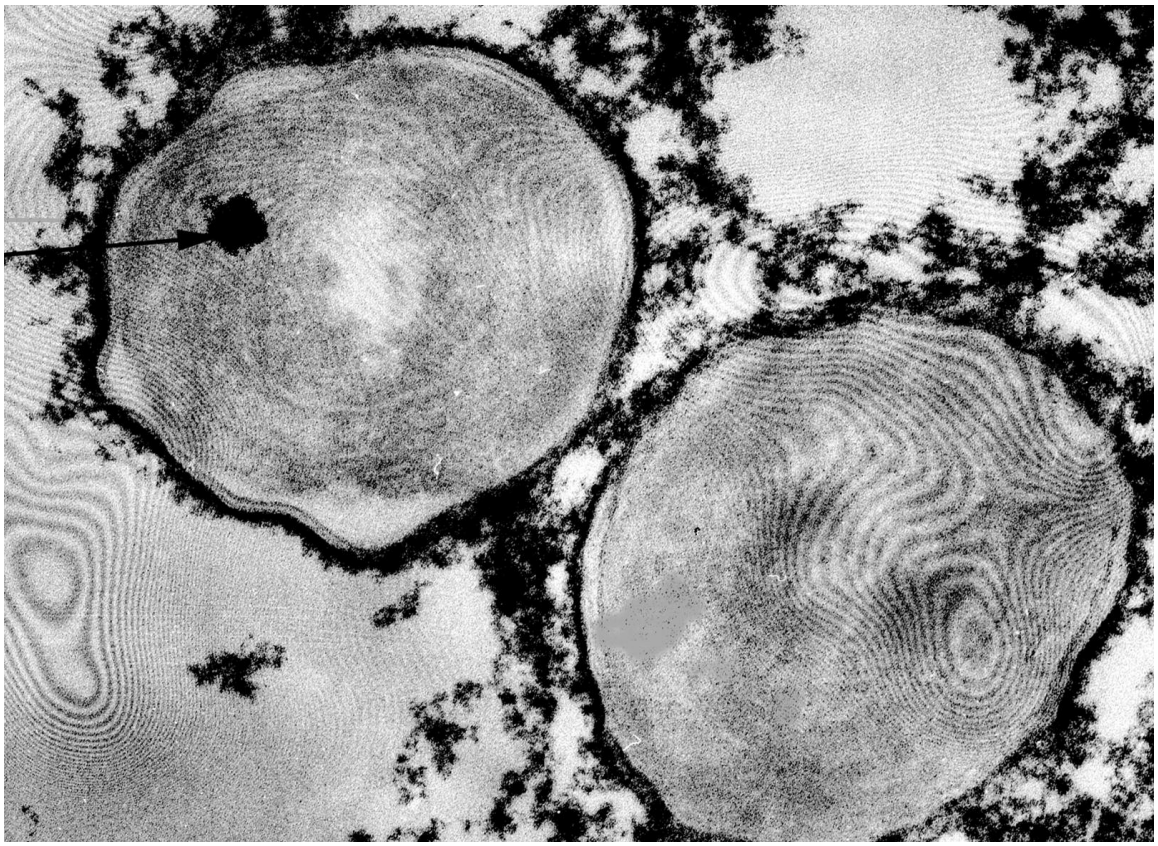


Figure 6. Thin section of *Arthrobacter globiformis,* Electron dense bodies are PPB's magnified 30,000X. Arrow points to PPBs.

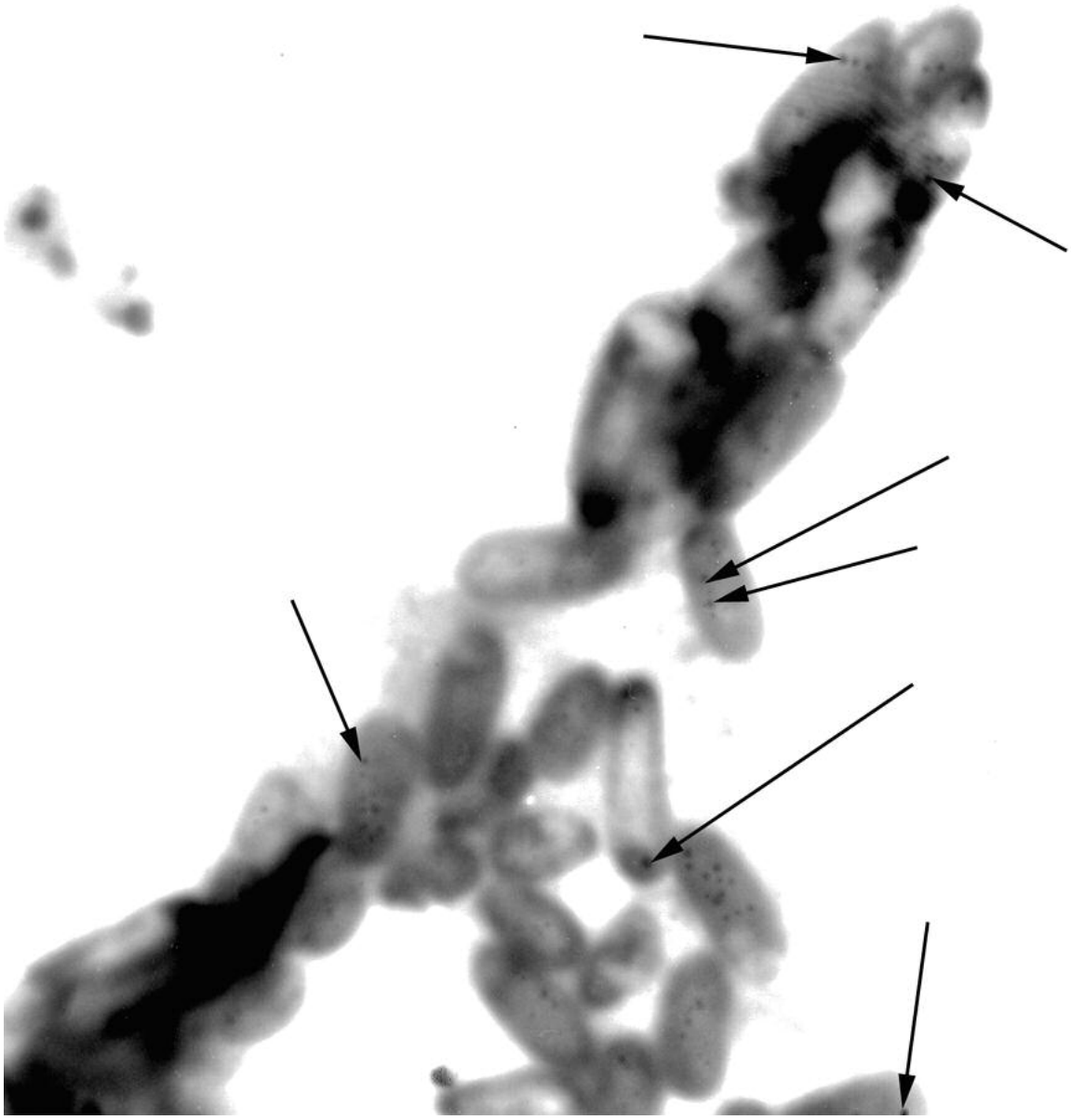


Figure 7. Air dried samples of *Arthrobacter globiformis,* Electron dense bodies are PPB's magnified 20,000X. Arrow points to PPBs.

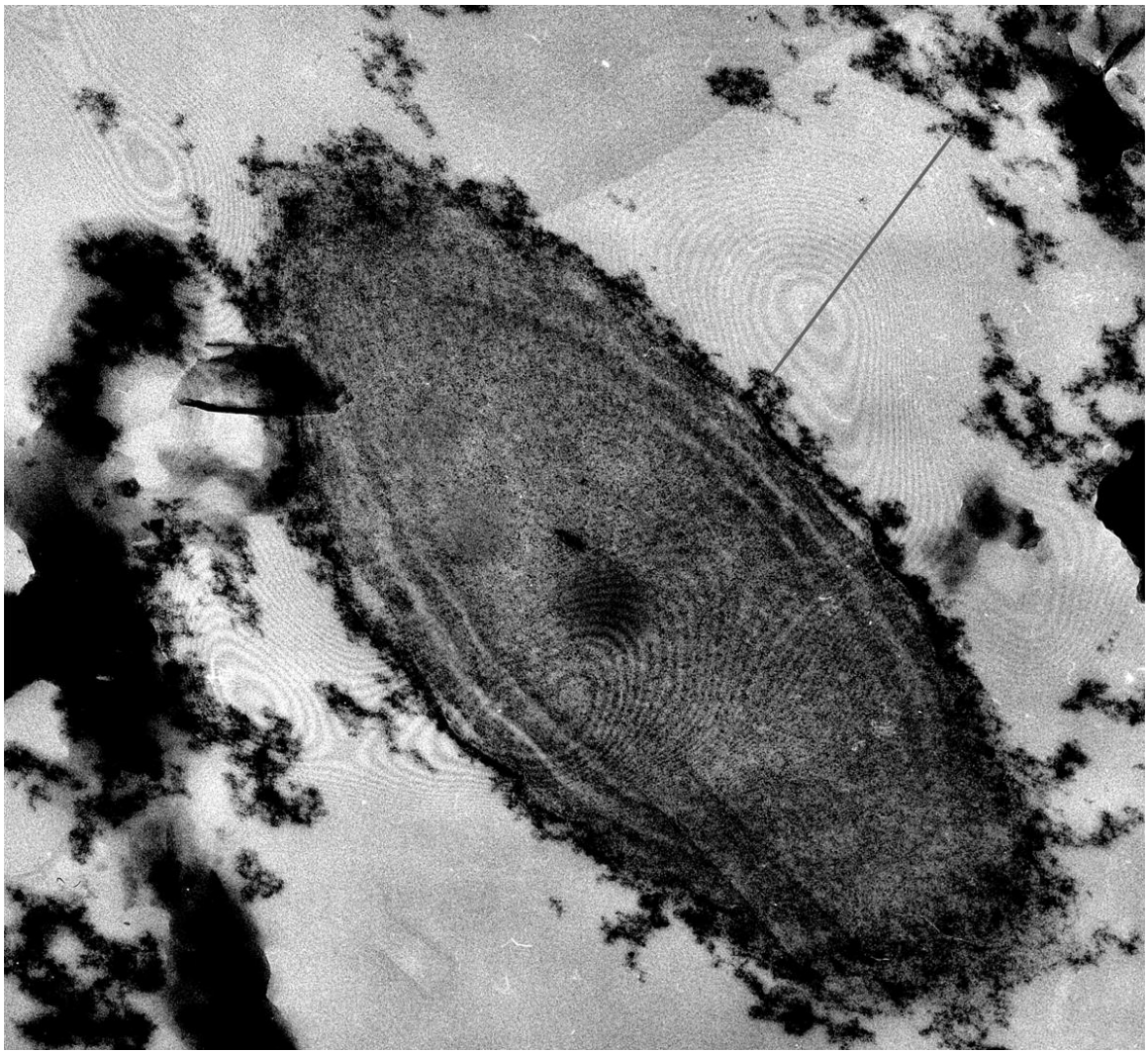


Figure 8: Air dried samples of *Pseudomonas aeruginosa,* Electron dense bodies are PPB's magnified 40,000X. Arrow points to PPBs.

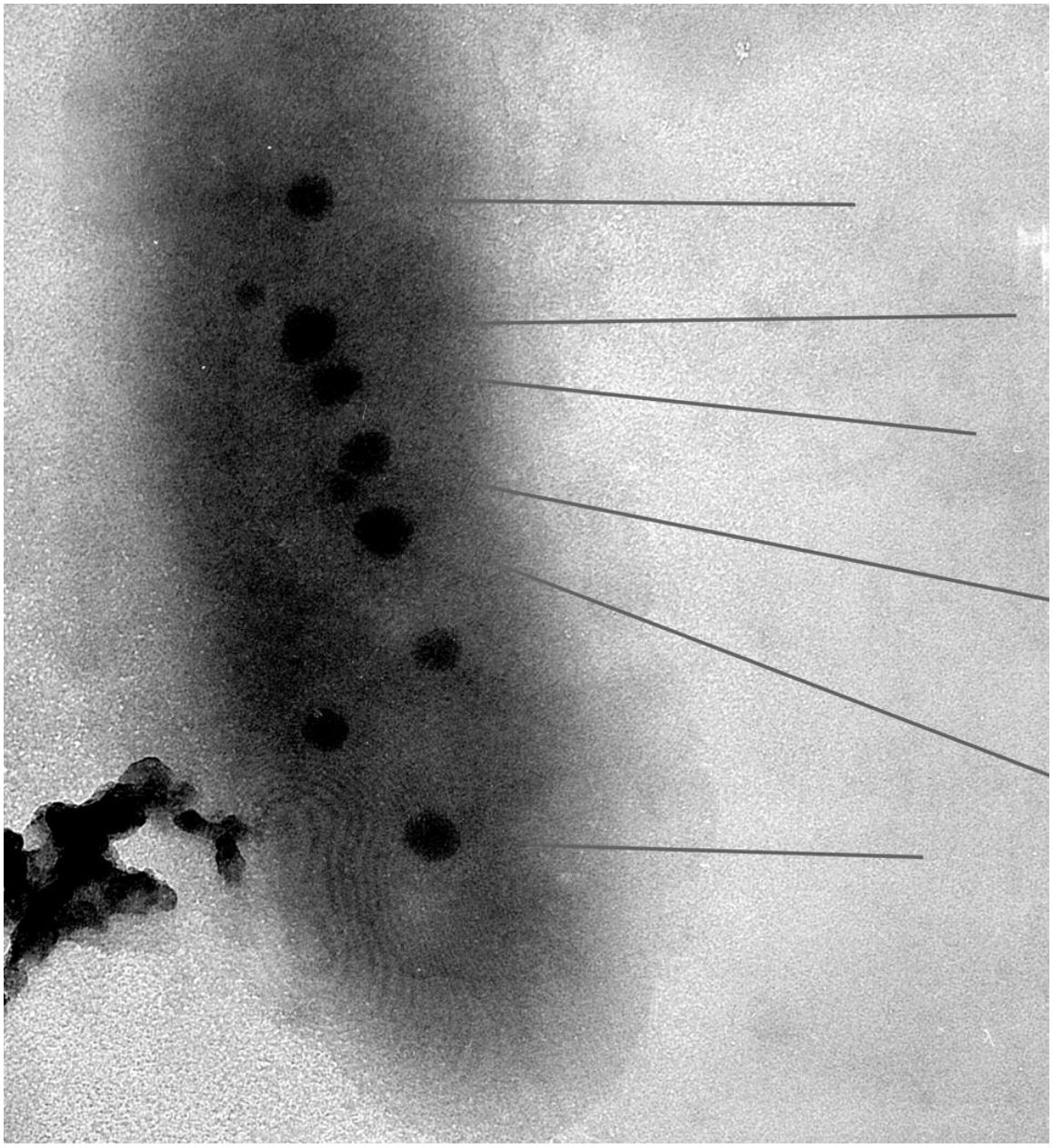


Figure 9: Air dried samples of *Escherichia coli,* Electron dense bodies are PPB's magnified 40,000X. Arrow points to PPBs.

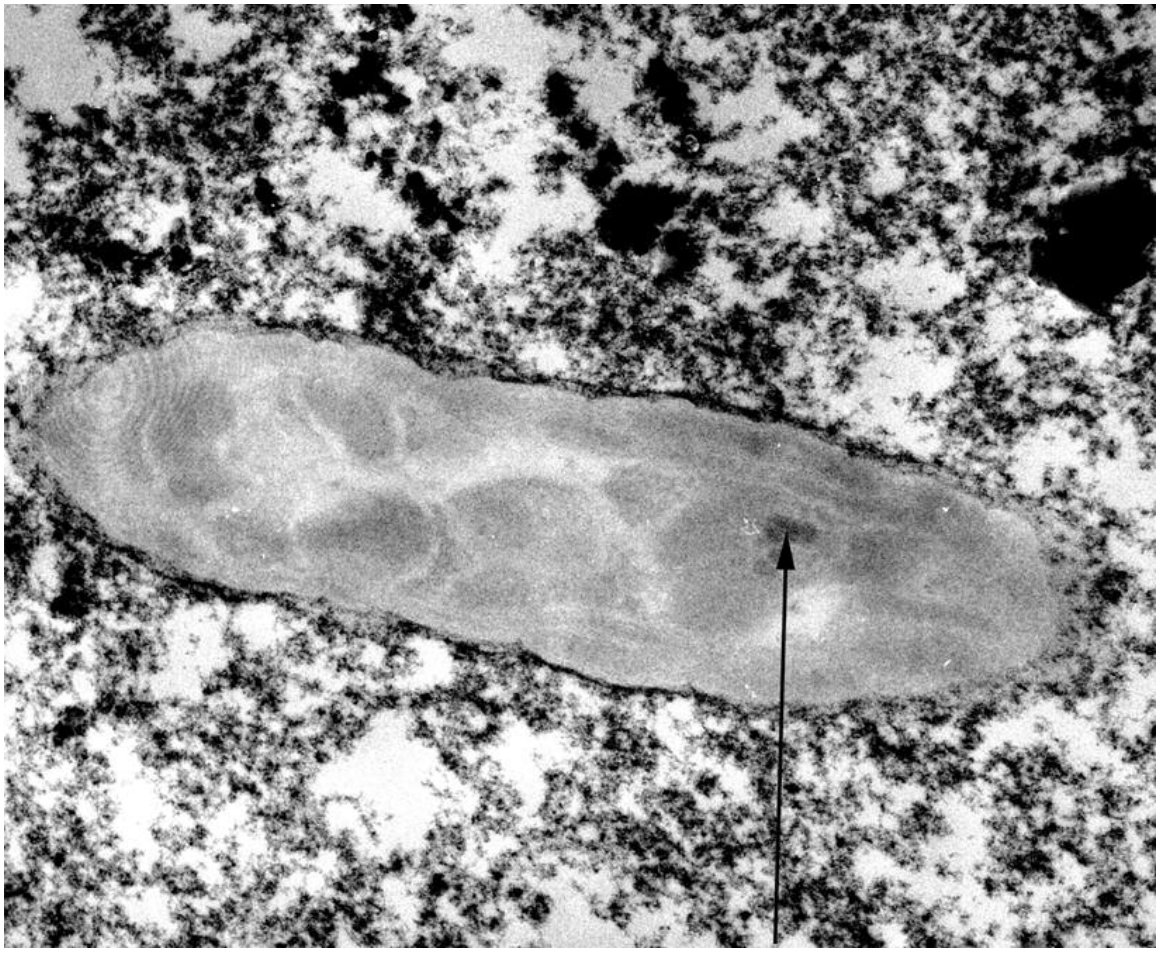


Figure 10: Air dried samples of *Sacharomyces cerevvisiae.* Electron dense body is PPB's magnified 40,000X. Arrow points to PPBs.

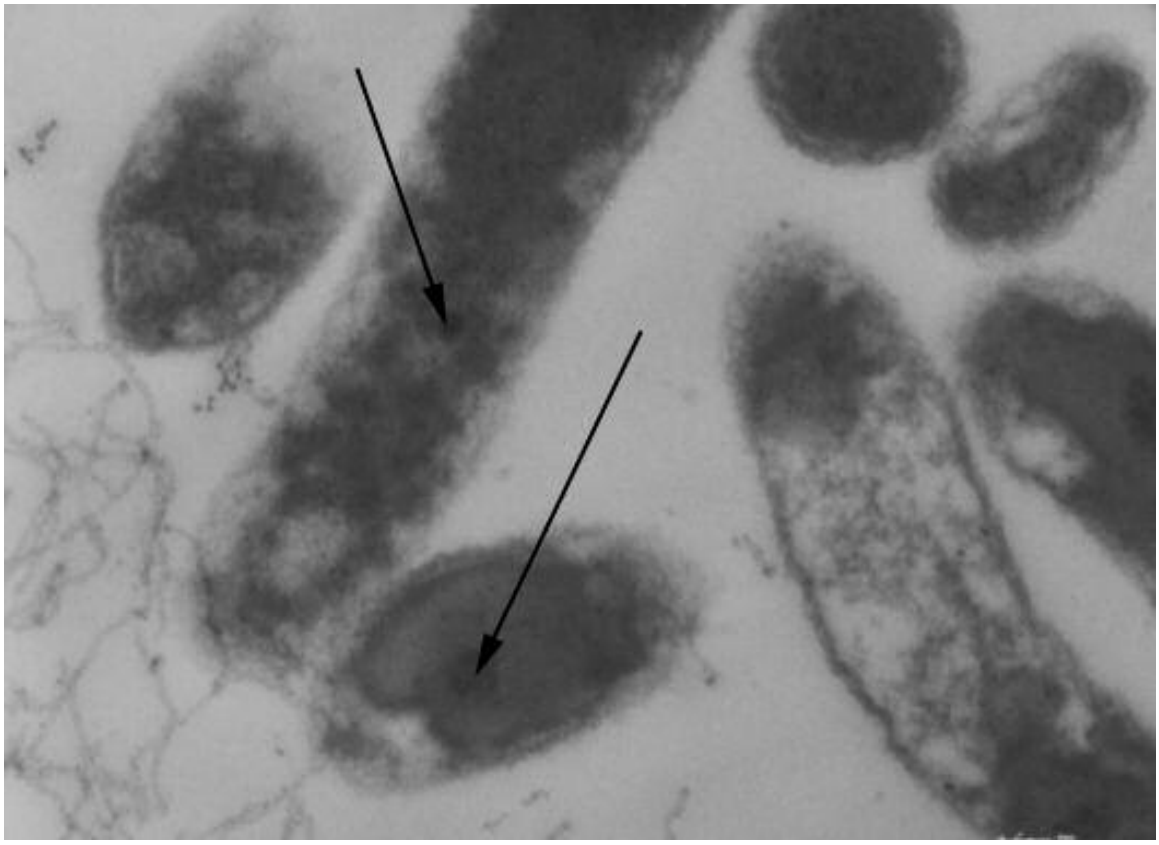


Figure 11: Air dried samples of *Rhodotorula rubra,* Electron dense body is PPB's magnified 40,000X. Arrow points to PPBs.

Cyanobacteria

Cyanobacteria are aquatic and photosynthetic, that is, they live in the water, and can manufacture their own food. Because they are bacteria, they are quite small and usually unicellular, though they often grow in colonies large enough to see. They have the distinction of being the oldest known fossils, more than 3.5 billion years old, in fact! It may surprise you then to know that the cyanobacteria are still around; they are one of the largest and most important groups of bacteria on earth.

Many Proterozoic oil deposits are attributed to the activity of cyanobacteria. They are also important providers of nitrogen fertilizer in the cultivation of rice and beans. The cyanobacteria have also been tremendously important in shaping the course of evolution and ecological change throughout earth's history. The oxygen in the atmosphere that we depend on was generated by numerous cyanobacteria during the Archaean and Proterozoic Eras. Before that time, the atmosphere had a very different chemistry, unsuitable for life, as we know it today.

The other great contribution of the cyanobacteria is the origin of plants. The chloroplast with which plants make food for themselves is actually a cyanobacterium living within the plant's cells. Sometime in the late Proterozoic, or in the early Cambrian, cyanobacteria began to take up residence within certain eukaryote cells, making food for the eukaryote host in return for a home. This event is known as endosymbiosis, and is also the origin of the eukaryotic mitochondrion.

Because they are photosynthetic and aquatic, cyanobacteria are often called "blue-green algae.” This name is convenient for talking about organisms in the water that make their own food, but does not reflect any relationship between the cyanobacteria and other organisms called algae. Cyanobacteria are relatives of the bacteria, not eukaryotes, and it is only the chloroplast in eukaryotic algae to which the cyanobacteria are related.

Though cyanobacteria do not have a wide diversity of form, and thought they are microscopic, they are rich in chemical diversity. Cyanobacteria once known as blue-green algae get their name from the bluish pigment phycocyanin, which they use to capture light in the process of photosynthesis. However, not all "blue-green" bacteria are blue; some common forms are red or pink from the pigment phycoerythrin. These bacteria are often found growing on greenhouse glass, or around sinks and drains. The Red Sea gets its name from occasional blooms of a reddish species of *Oscillatoria*, and African flamingos get their pink color from eating *Spirulina*. Whatever their color, cyanobacteria are photosynthetic, and so can manufacture their own food. The term "algae" merely refers to any aquatic organisms capable of photosynthesis, and so applies to several groups.

Cyanobacteria are very important organisms for the health and growth of many plants. They are one of very few groups of organisms that can convert inert atmospheric nitrogen into an organic form, such as nitrate or ammonia. It is these "fixed" forms of nitrogen that plants need for their growth, and must obtain from the soil. Fertilizers work the way they do in part because

they contain additional fixed nitrogen that plants can then absorb through their roots.

Nitrification cannot occur in the presence of oxygen, so nitrogen is fixed in specialized cells called heterocysts. These cells have an especially thickened wall that contains an anaerobic environment. Many plants, especially legumes, have formed symbiotic relations with nitrifying bacteria, providing specialized tissues in their roots or stems to house the bacteria, in return for organic nitrogen. This has been used to great advantage in the cultivation of rice, where the floating fern *Azolla* is actively distributed among the rice paddies. The fern houses colonies of the cyanobacterium *Anabaena* in its leaves, where it fixes nitrogen. The ferns then provide an inexpensive natural fertilizer and nitrogen source for the rice plants when they die at the end of the season. Cyanobacteria also form symbiotic relationships with many fungi, forming complex symbiotic "organisms" known as lichens.

All cells have an exceptional capacity to take up elements from a solution against a concentration gradient. With chlorine being totally rejected and

sodium weakly rejected (in seawater), all other elements are concentrated to some extent. Organismic groups concentrate these elements to various degrees. Since concentration factors seem not to be linked to essentiality, calcium, magnesium, lead, strontium, thallium, titanium, zirconium are strongly concentrated (Bowen, 1966). Microorganisms, specifically bacteria, fungi, and algae, exhibit such a capacity to accumulate cations that that they have been used extensively in studies which investigate biochemical, physiological and ultrastructural changes induced by environmental cations, pesticides and many other materials which are suspected to produce toxic effects. Recent considerations include using these organisms for removal of valuable and threatening ions (Lobban *et al.* 1985, Zimnik and Sneddon, 1988; Mallick and Rai, 1993).

The cyanobacterium *Synechoccus leopoliensis* of the Order Chroococcales is also asymbiotic and aerobic. A small cell with a length of approximately 4μm, this species of cyanobacterium is very common in the environment and has been the subject of extensive research (Jensen and Rachlin, 1984; Lawry and Jensen, 1986; Tang, 1993).

## Gram Positive Bacteria

### The Gram-Positive Cell Wall

A bacterial cell wall composed of a dense layer of peptidoglycan and teichoic acids. Gram-positive bacteria are those that retain the initial dye crystal violet during the Gram stain procedure and appear purple when observed through the microscope. Common gram-positive bacteria of medical importance include *Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus aureus, Enterococcus faecalis,* and *Clostridium* species.

Composition:

In electron micrographs, the gram-positive cell wall appears as a broad, dense wall 20-80 nm thick and consisting of numerous interconnecting layers of peptidoglycan. Chemically, 60 to 90% of the gram-positive cell wall is peptidoglycan. Interwoven in the cell wall of gram-positive are teichoic acids. Teichoic acids extend through and beyond the rest of the cell wall and are composed of polymers of glycerol, phosphates, and the sugar alcohol ribitol. Some teichoic acids have lipids attached and are called lipoteichoic acids. The outer surface of the peptidoglycan is studded with proteins that differ with the strain and species of the bacterium.

Function:

1. The peptidoglycan in the gram-positive cell wall prevents osmotic lysis *(def)*. The techoic acids probably help make the cell wall stronger.

2. In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with

human cells. These unique molecules are called pathogen-associated molecular patterns. Peptidoglycan and teichoic acids from the cell wall of gram-positive bacteria, binds to pattern-recognition receptors on a variety of defense cells of the body and triggers innate immune defenses such as inflammation, fever, and phagocytosis.

4. The peptidoglycan and teichoic acids also activate the alternative complement pathway and the lectin pathway, defense pathways that play a variety of roles in body defense.

5. The surface proteins in the bacterial peptidoglycan, depending on the strain and species, carry out a variety of activities, including:

- functioning as enzymes;
- serving as adhesins (allowing the bacterium to adhere intimately to host calls and other surfaces in order to colonize and resist flushing ); functioning as invasins (allowing some bacteria to penetrate host cells); and/or
- aiding certain bacteria in resisting phagocytic destruction.

Gram Negative Bacteria

A bacterial cell wall composed of a thin, inner layer of peptidoglycan and an outer membrane of phospholipids, lipopolysaccharide, and lipoprotein. During the Gram stain procedure, the gram-negative cell wall enables the bacterium to become decolorized and pick up the pink counterstain safari.

Common Gram-negative bacteria of medical importance include *Salmonella* species, *Shigella* species, *Neisseria gonorrhoeae, Neisseria meningitidis, Hemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Proteus* species, and *Pseudomonas aeruginosa*.

In electron micrographs, the gram-negative cell wall appears multilayered.

It consists of:

I. An Inner Membrane

That is a thin, inner wall composed of peptidoglycan (2-3 nm thick) .Its composition: 2-3 layers of peptidoglycan. Chemically, only 10 to 20% of the gram-negative cell wall is peptidoglycan. The function of the peptidoglycan is to prevent osmotic lysis.

II. An Outer Membrane

Is composed of a lipid bilayer about 7 nm thick composed of phospholipids, lipoproteins, lipopolysaccharides (LPS), and proteins. Phospholipids are located mainly in the inner layer of the outer membrane, as are the lipoproteins that connect the outer membrane to the peptidoglycan. The lipopolysaccharides, located in the outer layer of the outer membrane, consist of a lipid portion called lipid A embedded in the membrane and a polysaccharide portion extending outward from the bacterial surface. The outer membrane also contains a number of proteins that differ with the strain and species of the bacterium.

The outer membrane, like the cytoplasmic membrane discussed next, is semipermeable and acts as a coarse molecular sieve. Many small molecules may pass through due to pores running through the membrane. These pores are composed of proteins called porins.

The functions:

1. Because of its semipermeable nature, the outer membrane helps retain certain enzymes and prevents some toxic substances, *e.g*., penicillin G and lysozyme, from entering.

2. The LPS from the outer membrane of the gram-negative cell wall is thought to add strength to the outer membrane, in a manner similar to the glycopeptides and teichoic acids of the gram-positive cell wall.

3. In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with human cells. These unique molecules are called pathogen-associated molecular patterns. LPS, unique to the cell wall of gram-negative bacteria, binds to pattern-recognition receptors on a variety of defense cells of the body and triggers innate immune defenses such as inflammation, fever, and phagocytosis.

4. The surface proteins in the outer membrane, depending on the strain and species, carry out a variety of activities, including: functioning as enzymes; serving as adhesins, allowing the bacterium to adhere intimately to host cells

and other surfaces in order to colonize and resist flushing; functioning as invasions, allowing some bacteria to penetrate host cells aiding certain bacteria in resisting phagocytic destruction.

III. The Periplasm

The periplasm is the gelatinous material between the outer membrane, the peptidoglycan, and the cytoplasmic membrane. It contains enzymes for nutrient breakdown as well as binding proteins to facilitate the transfer of nutrients across the cytoplasmic membrane.

Eukaryotes

Yeasts

Fungi include yeasts, molds, and fleshy fungi. They are eukaryotic with rigid cell walls; they are chemoheterotrophs (require organic compounds for both carbon and energy sources). Yeasts obtain nutrients as saprophytes (living

off of decaying matter) or as parasites (living off of loving matter). Of the over 100,000 species of fungi, only about 100 species are pathogenic for animals. They play a major role in the recycling of nutrients by their ability to cause decay and are used by industry to produce a variety of useful products. However, they also cause many undesirable economic effects such as the spoilage of fruits, grains, and vegetables, as well as the destruction of unpreserved wood and leather products.

1. Yeast morphology

a. Yeast are unicellular fungi that usually appear as oval cells 1-5 μm wide by 5-30 μm long.

b. They have typical eukaryotic structures

c. They have a thick polysaccharide cell wall.

In order to protect against infection, one of the things the body must initially do is detect the presence of microorganisms. The body does this by recognizing molecules unique to microorganisms that are not associated with human cells.

These unique molecules are called pathogen-associated molecular patterns. Components of the yeast cell wall bind to pattern-recognition receptors on a variety of defense cells of the body and triggers innate immune defenses such as inflammation, fever, and phagocytosis.

d. They are facultative anaerobes, organisms that grow with or without oxygen. They get their energy through aerobic respiration as well as fermentation.

2. Reproduction of yeasts

a. Yeasts reproduce asexually by a process called budding. A bud is formed on the outer surface of the parent cell as the nucleus divides. One nucleus migrates into the elongating bud. Cell wall material forms between the bud and the parent cell and the bud breaks away.

b. A few yeasts, such as *Candida albicans*, also produce clusters of asexual reproductive spores called blastospores, Asexual reproductive spores of yeast produced through budding, and thick-walled survival

spores called chlamydospores, thick-walled survival spores produced by the yeast *Candida*.

c. Yeasts can also reproduce sexually by means of sexual spores called ascospores, which result from the fusion of the nuclei from two cells followed by meiosis. Sexual reproduction is much less common than asexual reproduction but does allow for genetic recombination.

Cell Wall and Sheath

The cyanobacteria all have a sheath as do most of the gram +, gram-, and eukaryotic cells. The sheath of the cyanobacteria is peripheral to the four layers of the cell wall. It is probably composed of polysaccharides. The four layered cell wall of an electron transparent layer just outside the plasma membrane, followed by the electron dense mucopolymer layer. Outside the mucopolymer layer is another layer that is also electron transparent, it is layer three. Layer four resembles a unit membrane. All the layers combined are about 10nm thick. In some of the organisms the mucopolymer layer is thicker. In some organisms there are pores present in the cell wall. The outer membrane in some species acts as a barrier to prevent the passage of certain

enzymes in the sheath. In *Gloeocapsa alpicola* additional layers are found in the cell wall (Jensen & Sicko, 1972).

Polyphosphate Bodies

Polyphosphate bodies are found in some cells in all normal cultures. In cell grown under unfavorable environmental conditions with phosphorus present they grow in increased numbers and also in increased size. They appear either as electron dense inclusions or dense inclusions with a porous appearance (Jensen, 1968). They are generally poorly infiltrated in the embedding process and thus will very often fall out of the sections. It is probable that they have a lipid or protein component in addition to the polyphosphate and other elements like C, O, K, Mg, and Ca. These bodies function as a phosphorus reserve, as an energy source and as a detoxifying mechanism by trapping toxic heavy metals (Baxter & Jensen, 1980b; Jensen et al., 1982b)

## Heavy Metals

### Heavy Metal Toxicity

Metals can either be considered essential or toxic to living organisms. Iron for instance is a component of hemoglobin and other important enzymes; iron deficiency can lead to disease. Other metals even in trace amounts can lead to disease. Copper and zinc are nutrients required for the metabolism in small amounts, yet in larger amounts these metals are toxic. Cadmium and mercury, on the other hand have no known biological functions and are considered consistently toxic at all concentrations (Stokes, 1983; Reed and Gadd, 1989). Metals like Cd, Hg, Pb, Cu, Zn, and Al are commonly encountered as environmental pollutants and are often referred to as heavy metals.

Distributed throughout the earth's crust, cadmium is one of the most toxic metals (Reed and Gadd, 1989). It has become a pollutant in the past few years due to increased excavations and industrial consumption. Since it is

commonly present in water, food and tobacco, as well as in soil and the atmosphere, Cd is exposed to living systems and seems to be readily absorbed by the body (Merian, 1990) and has been shown to be very harmful to human beings; the target tissues include the kidney and the liver.

In New York and New Jersey Harbor Estuary area, a high concentration of Cd, along with Cu, Pb, Ni, Ag, and Zn, in water and sediment categorized them as toxins with respect to their effect on marine life (Squibb et al., 1991). In aquatic systems, algal diversity and productivity have decreased as a general result of pollution by metals (Rai et al., 1981). It has been reported that a very toxic metal such as cadmium generally will inhibit cell growth.

Rachlin et al. (1982a) studied the effect of eight different metals on the overall cell volume of *Plectonema boryanum.* Cells were exposed to 10 ppm of $Mn^{2+}$, $Zn^{2+}$, $Hg^{2+}$, $Cd^{2+}$, $Pb^{2+}$, $Cu^{2+}$, $Ni^{2+}$, and, $Co^{2+}$. While $Mn^{2+}$, $Zn^{2+}$, $Hg^{2+}$, and $Cd^{2+}$, produced no change in the overall cell volumes, $Pb^{2+}$, $Cu^{2+}$, produced significant increase in cell volume. $Ni^{2+}$, and, $Co^{2+}$, produced a significant decrease in volume.

Although aluminum is generally regarded as a non-essential element, studies show that, at low concentrations, the metal affords advantageous effects on certain plants. Foy (1974) traced the benefits of aluminum, he reported on the catalytic activity in photosynthesis and its inducement to germination in various species. The effective concentrations of aluminum generally ranged from 0.1 ppm to 5 ppm with large tubers responding positively to 20 ppm. In a later paper Foy et al. (1978) speculated that while the mechanisms of aluminum activity in plants were not yet clear, the responses that were observed may not necessarily result from the metal's effects *per se*. It may rather have some kind of a physiological effect on the plant such as displacing bound iron to overcome a deficiency, promoting phosphorus uptake negative charges on the cell wall, and retarding root deterioration in the presence of calcium deficiency by slowing the growth rate to prevent calcium depletion.

There are many studies on the toxicological effects of heavy metals, particularly cadmium, zinc, lead, and copper (Jensen et al., 1982a; Jensen et

al., 1982b; Rachlin et al., 1984; Rachlin et al., 1985; Andersson, 1992; de Lima and Copeland, 1994; Lindberg and Griffiths, 1993). The results of these studies have greatly clarified an organism's growth, photosynthetic, biochemical physiological and morphological responses to these pollutants. Yet, many of the specifics of metal toxicity remain unclear.

Mercury is reportedly one of the most toxic of the heavy metals (Bowen, 1966; Hongve et al., 1980). The report that mercury intensified fluorescence in the cyanobacterium, *Spirulina platensis* (Murthy et al., 1980), points not only to a localization of the metal in the phycobilisomes, but also innumerable changes resulting from the inhibition of Photosystems I and II.

The term *heavy metals* have been widely used, although there has been very little description given to these elements aside from their capacity to induce toxicological responses, particularly at higher concentrations. Niebor and Richardson (1980), noting the lack of consistency in definition and classification of heavy metals among zoologists, botanists, and other scientists, suggested a classification of metals into three categories. The categories, (1) class A (oxygen–seeking, i.e., aluminum, beryllium,

calcium), (2) class B (nitrogen/sulphur-seeking, i.e.,) and (3) borderline (intermediate, i.e., arsenic, iron, lead), would reflect not only the elements' biological activity and toxicity, but also their chemistry. The researchers go on to substantiate their proposal through a detailed discussion of metal-ligand complex formation in solution.

The term 'trace metals' has been used in biology for a few years now. It refers to metals that are essential microelements which may limit growth, if insufficient supplies are found, yet they prove to be toxic if found in great concentrations.Despite these and other proposals for terminology, the term 'heavy metals' predominates the literature, and will be used throughout the current project, mainly in order to reflect their toxicity.

Heavy metals differ from other aquatic pollutants in that, as apposed to pesticides and herbicides they are not biodegradable. They can persist and accumulate in the sedimentary sinks from where they can be re-released back into the water column. This can alter the density, diversity, structure,

and species composition of aquatic communities (Moore and Ramamoorthy, 1984).

Heavy metals are being added to bodies of water from industrial effluents, sewage, atmospheric fallout, geological weathering, and mining and drilling. Once in the water the metal concentration is lowered, after the initial dilution, by precipitation, adsorption and absorption by aquatic organisms. Precipitation occurs if the concentration of the metal is higher than the solubility of the least soluble compound that can be formed between the metal and ions in the water (Bryan, 1971).

Adsorption of metallic ions at the cell surface and their fate

It is widely known that soluble metals will inevitably reach and interact with the broad surfaces of bacteria (Beveridge, 1989). This contact and interaction are mainly due to the high surface area to volume ratio noted among small cells. This design is in order to benefit the bacteria since they must receive all of their nutrients through diffusion. The cells faculty for

diffusion calls for uptakes of nearly all solutes, beneficial or harmful, which are small enough to cross the cell's barrier. As a result, the physical and biochemical nature of the cell wall is a determinant of ionic uptake.

Metal uptake may be affected by the age and physiological state of the organisms, as well by the aerobic and anaerobic conditions (Silverberg et al., 1976). It can also be influenced by temperature, salinity, water hardness, the presence of other pollutants, the sediment type and the microbial mass present.

## Wall Penetration

The action of the cell surface as far as the cell the metal ion binding is concerned it is likened to a commercial ion-exchange resin, (Lindemann et al., 1990; Remacle, 1990). According to the Langmurian theory of adsorption (Volesky, 1990), a polar or nonpolar surface, will preferentially adsorb the more polar or nonpolar component of a solution in a

nonpolar/polar solvent. Some of the ions will adhere while others will flow back to the solution, equilibrium being established when the adsorbing surface is covered. This is the process by which cations, including heavy metals, become associated with the cell wall of phytoplankton. When metallic ions from the aquatic environment reach the cell surface of an organism, binding can occur through displacement of hydrogen ions (Ramelow et al., 1991) or by complexation with anionic ligands (Beveridge et al., 1982; Remacle, 1990).

Once ions have become bound up at the cell surface they may be immobilized through precipitation by the same ligands, or they may enter the cytoplasm by either passive or active diffusion. (Mullen et al.1989).

## The Fate of Intercellular Heavy Metals

The process by which heavy metal enters the cell has been outlined by Trevors and Cotter 1990, and Fiore (1994). The process is a two-step one; initially there is a rapid yet passive uptake of the heavy metal. This occurs

with out any temperature influence. This binding, which occurs soon after the organism encounters the ions, is followed by a slow phase during which heavy metals are incorporated into the cell itself. The second process is both dependent on the temperature and the metabolism of the organism. Once the heavy metals have been incorporated into the cells they may localize themselves in any sector including the nucleus, the cell wall, the vacuoles, or the polyphosphate bodies.

Aluminum

History:

(L. *alumen*: alum) The ancient Greeks and Romans used alum as an astringent and as a mordant in dyeing. In 1761 de Morveau proposed the name alumine for the base in alum, and Lavoisier, in 1787, thought this to be the oxide of a still undiscovered metal.

Wohler is generally credited with having isolated the metal in 1827, although an impure form was prepared by Oersted two years earlier. In 1807, Davy proposed the name *aluminum* for the metal, undiscovered at that time, and later agreed to change it to aluminum. Shortly thereafter, the name

aluminum was adopted to conform with the "ium" ending of most elements, and this spelling is now in use elsewhere in the world.

Aluminium was also the accepted spelling in the U.S. until 1925, at which time the American Chemical Society officially decided to use the name aluminum thereafter in their publications.

Sources:

The method of obtaining aluminum metal by the electrolysis of alumina dissolved in cryolite was discovered in 1886 by Hall in the U.S. and at about the same time by Heroult in France. Cryolite, a natural ore found in Greenland, is no longer widely used in commercial production, but has been replaced by an artificial mixture of sodium, aluminum, and calcium fluorides.

Aluminum can now be produced from clay, but the process is not economically feasible at present. Aluminum is the most abundant metal to be found in the earth's crust (8.1%),

but is never found free in nature. In addition to the minerals mentioned above, it is also found in granite and in many other common minerals.

Properties:

Pure aluminum, a silvery-white metal, possesses many desirable characteristics. It is light, it is nonmagnetic and nonsparking, stands second among metals in the scale of malleability, and sixth in ductility.

Uses:

It is extensively used for kitchen utensils, outside building decoration, and in thousands of industrial applications where a strong, light, easily constructed material is needed.

Although its electrical conductivity is only about 60% that of copper, it is used in electrical transmission lines because of its light weight. Pure aluminum is soft and lacks strength, but alloyed with small amounts of copper, magnesium, silicon, manganese, or other elements impart a variety of useful properties.

These alloys are of vital importance in the construction of modern aircraft and rockets. Aluminum, evaporated in a vacuum, forms a highly reflective coating for both visible light and radiant heat. These coatings soon form a thin layer of the protective oxide and do not deteriorate as do silver coatings.

They are used to coat telescope mirrors and to make decorative paper, packages, and toys.

Compounds:

The compounds of greatest importance are aluminum oxide, the sulfate, and the soluble sulfate with potassium (alum). The oxide, alumina, occurs naturally as ruby ($Al_2O_3$), sapphire, corundum, and emery, and is used in glassmaking and refractories.

According to one hypothesis, organisms through their evolvement in biochemical stages they chose substances that were found in abundance in the earth's crust and were readily soluble in the anaerobic environment (Driscoll and Scherer, 1990). Aluminum is the most abundant metal in the earth's crust, but was, no doubt, available to anaerobic life forms. This may explain why this abundant element is not only non-essential, but is also found in very limited concentrations in living tissues, occurring as complex silicates (clay), oxides (bauxite), and sulfates.

This metal is too reactive to occur free in nature (Haug, 1984). It occurs, instead, as complex silicates (clay), oxides (bauxite), and sulfates. In natural waters, the metal can be found in two forms: soluble salts and colloidal compounds. It has been found that the concentrations found in unpolluted waters range from 0.10 mg/L to 10 mg/L (Galvin, 1991). There are rivers in Spain that report having ranges between 0.09mg/L and 1.86 mg/L, while in the rivers of North America the ranges are between 0.012 mg/L and 2.250 mg/L (Galvin, 1991). In 1988 the Environmental Protection Agency (EPA) published water quality criteria for the protection of aquatic organisms exposed to aluminum. This document recommends that within any four day period the concentration of aluminum should not exceed 8.7 μg/L more than once every three years where the pH is between 6.5 and 9.0 (Gostomski, 1990).

In general, aluminum is trivalent, though there is evidence that it may occur in a monovalent or bivalent state. Its oxide, $Al_2O_3$ , is amophoteric, acting as a base to form salts with acids, or as a weak acid to form salts with strong alkalides.

Aluminum is available for biological interaction only when it becomes mobilized in the soil, and even then, its activity depends on the pH, the amount of metal that is available and the inorganic and organic complexing agents that are involved. According to Haug (1984), aluminum becomes soluble at pH ≥ 5.0, at concentrations between 1 ppm and 30 ppm. The values are represntative of aluminum concentrations found in soils.

Even though aluminum is regarded as a non-essential element, there is some consideration that, at low concentrations, the metal exerts beneficial effects on some plants (Foy *et al*., 1978; Kinraide, 1993). Foy et al. 1978 proposed that the metal could possibly increase iron solubility and availability in certain soils, promote the uptake of phosphorus by block negative charges on the cell wall, or protect calcium-deficient plants from root deterioration by slowing down their growth and preventing depletion of the nutrient.

Cadmium

History:

(L. *cadmia*; Gr. *kadmeia* - ancient name for calamine, zinc carbonate)

Discovered by Stromeyer in 1817 from an impurity in zinc carbonate. Cadmium most often occurs in small quantities associated with zinc ores, such as sphalerite (ZnS). Greenockite (CdS) is the only mineral of any consequence bearing cadmium. Almost all cadmium is obtained as a by-product in the treatment of zinc, copper, and lead ores. It is a soft, bluish-white metal, which is easily cut with a knife. It is similar in many respects to zinc. In 1927 the International Conference on Weights and Measures redefined the meter in terms of the wavelength of the red cadmium spectral line (i.e. 1m = 1.553,164.13 wavelengths). This definition has been changed (see Krypton).

Uses:

Cadmium is a component of some of the lowest melting alloys; it is used in bearing alloys with low coefficients of friction and great resistance to fatigue; it is used extensively in electroplating, which accounts for about 60% of its use. It is also used in many types of solder, for standard E.M.F.

cells, for Ni-Cd batteries, and as a barrier to control nuclear fission. Cadmium compounds are used in black and white television phosphors and in blue and green phosphors for color TV tubes. It forms a number of salts, of which the sulfate is most common; the sulfide is used as a yellow pigment. Cadmium and solutions of its compounds are toxic.

Handling:

Failure to appreciate the toxic properties of cadmium may cause workers to be unwittingly exposed to dangerous fumes. Silver solder, for example, which contains cadmium, should be handled with care. Serious toxicity problems have been found from long-term exposure and work with cadmium plating baths. Exposure to cadmium dust should not exceed 0.01 $mg/m^3$ (8-hour time-weighted average, 40-hour week). The ceiling concentration (maximum), for a period of 15 min, should not exceed 0.14 $mg/m^3$. Cadmium oxide fume exposure (8-hour, 40-hour week) should not exceed 0.05 $mg/m^3$, and the maximum concentration should not exceed 0.05 $mg/m^3$. These values are presently being restudied and recommendations have been made to reduce the exposure.

Cadmium is generally associated with zinc in carbonate and sulfide ores. It is obtained as a by-product in the refining of copper, lead and zinc. In industry cadmium is used for electroplating and for the production of pigments, plastic stabilizers and batteries (Moore and Ramamoorthy, 1984). Precipitation effectively removes cadmium from the atmosphere. It's the time that the element spends in the oceans that seems to vary, $10^4$ to $10^5$ (Blistrieri *et al*., 1981 cit. by Livett, 1988).

Cadmium is different than copper, it is believed to have nonnutritive value for algae or other organisms. Cadmium has been reported to inhibit photosynthetic $^{14}CO_{2,}$ uptake (Stratton and Corke, 1979) and calcium uptake (Pick et al., 1986a; 1986b). It would seem to decrease algal growth rates (Rachlin et al., 1982b) and cause loss of motility in *Euglena gracilis* (Fennikoh et al., 1978). Cadmium also induces filament elongation and loss of cellular contents in the apical cells of the filaments in *Anabaena inaequalis* (Stratton and Corke, 1979).

In humans the most serious case of cadmium poisoning, called Itai-itai disease, was diagnosed in Japan from 1940-1960. Untreated waste from mines discharged into local rivers and heavily contaminated both the drinking water and rice fields. Patients suffering from the disease exhibited skeletal deformation and renal dysfunction. In addition epidemiological studies have established a causal link between cadmium exposure and cancer incidence (Moore and Ramamoorthy, 1984).

Copper

History:

(Latin *cuprum*, from the island of Cyprus) It is believed that copper has been mined for 5,000 years.

Properties:

Copper is reddish and takes on a bright metallic luster. It is malleable, ductile, and a good conductor of heat and electricity (second only to silver in electrical conductivity).

Sources:

Copper occasionally occurs natively, and is found in many minerals such as cuprite, malachite, azurite, chalcopyrite, and bornite.

Large copper ore deposits are found in the U.S., Chile, Zambia, Zaire, Peru, and Canada. The most important copper ores are the sulfides, the oxides, and carbonates. From these, copper is obtained by smelting, leaching, and by electrolysis.

Uses:

The electrical industry is one of the greatest users of copper. Iron's alloys -- brass and bronze -- are very important: all American coins are copper alloys and gunmetals also contain copper.

Copper has wide use as an agricultural poison and as an algaecide in water purification. Copper compounds, such as Fehling's solution, are widely used in analytical chemistry tests for sugar.

Copper is widely distributed throughout nature in a free state in sulfides, arsenides chlorides and carbonates. It is universally used in the electrical, construction and plumbing, and automotive industries (Moore and Ramamoorthy, 1984) and therefore, poses a potential threat to the environment through anthropogenic input. Copper is an essential component of the photosynthetic electron transport system and a component or cofactor of several (Kaplan et al., 1984). Trace amounts, therefore, are required for the metabolic processes of algae (Rai et al., 1981). Higher concentrations of copper are toxic, and copper in the form of copper sulphate has been widely used as an algicide to control undesirable algal growth. The dual role that copper has as both a micronutrient and toxicant has been demonstrated by Sandman and Boger (1980). They reported that *Skenedesmus acutus* acquired 0.1-1.0 μM of copper for optimum growth. Chlorophyll became bleached in Cu depleted cultures and growth decreased by 50%. Copper concentrations exceeding 10 μM were temporarily toxic leading to chlorophyll degradation and oxidation of lipids. There are other studies involving algae and there exposure to Cu. These studies determine that Cu will inhibit the growth and photosynthesis abilities of algae. The copper will also affect the cell permeability of the plasma membrane causing the loss of

organic matter (Steeman-Nielsen and Wium Andersen, 1971), the loss of potassium (Rai et al., 1981), and the uptake of essential elements and compounds such as manganese (Sunda and Huntsman et al.1981).

Manganese

History:

(L. *magnes*: magnet, from magnetic properties of pyrolusite; Itl. *manganese*, corrupt form of magnesia)

Recognized by Scheele, Bergman, and others as an element and isolated by Gahn in 1774 by reduction of the dioxide with carbon.

Sources:

Manganese minerals are widely distributed; oxides, silicates, and carbonates are the most common. The discovery of large quantities of manganese nodules on the floor of the oceans may become a source of manganese. These nodules contain about 24% manganese, together with many other elements in lesser abundance.

Most manganese today is obtained from ores found in Russia, Brazil, Australia, South Africa, Gabon, and India. Pyrolusite and rhodochrosite are among the most common manganese minerals. The metal is obtained by reduction of the oxide with sodium, magnesium, aluminum, or by electrolysis.

Properties:

It is gray-white, resembling iron, but is harder and very brittle. The metal is reactive chemically, and decomposes cold water slowly. Manganese is used to form many important alloys. In steel, manganese improves rolling and forging qualities, strength, toughness, and stiffness, wear resistance, hardness, and harden ability. With aluminum and antimony, and especially with small amounts of copper, it forms highly ferromagnetic alloys.

Manganese metal is ferromagnetic only after special treatment. The pure metal exists in four allotropic forms. The alpha form is stable at ordinary

temperature; gamma manganese, which changes to alpha at ordinary temperatures, is said to be flexible, soft, easily cut, and capable of being bent.

Uses:

The dioxide (pyrolusite) is used as a depolarizer in dry cells, and is used to "decolorize" glass that is colored green by impurities of iron. Manganese by itself colors glass an amethyst color, and is responsible for the color of true amethyst. The dioxide is also used in the preparation of oxygen and chlorine, and in drying black paints. The permanganate is a powerful oxidizing agent and is used in quantitative analysis and in medicine. Manganese is widely distributed throughout the animal kingdom. It is an important trace element and may be essential for utilization of vitamin B1.

Handling:

Exposure to manganese dusts, fume, and compounds should not exceed the ceiling value of 5 $mg/m^3$ for even short periods because of the element's toxicity level.

Nickel

History:

(German *Nickel*, Satan and from *kupfernickel*, Old Nick's copper) Cronstedt discovered nickel in 1751 in kupfernickel (niccolite).

Sources:

Nickel is found as a constituent in most meteorites and often serves as one of the criteria for distinguishing a meteorite from other minerals. Iron meteorites, or *siderites*, may contain iron alloyed with from 5 percent to nearly 20 percent nickel. Nickel is obtained commercially from pentlandite and pyrrhotite of the Sudbury region of Ontario, a district that produces about 30 percent of the world's supply of nickel. Other deposits are found in New Caledonia, Australia, Cuba, Indonesia, and elsewhere.

Properties:

Nickel is silvery white and takes on a high polish. It is hard, malleable, ductile, somewhat ferromagnetic, and a fair conductor of heat and electricity. It belongs to the iron-cobalt group of metals and is chiefly valuable for the alloys it forms.

Uses:

It is extensively used for making stainless steel and other corrosion-resistant alloys such as Invar(R), Monel(R), Inconel(R), and the Hastelloys(R). Tubing made of copper-nickel alloy is extensively used in making desalination plants for converting seawater into fresh water.

Nickel, used extensively to make coins and nickel steel for armor plates and burglar-proof vaults, and is also a component in Nichrome(R), Permalloy(R), and constantan.

Nickel gives glass a greenish color. Nickel plating is often used to provide a protective coating for other metals, and finely divided nickel is a catalyst for hydrogenating vegetable oils. It is also used in ceramics, in the manufacture of Alnico magnets, and in the Edison(R) storage battery.

Isotopes:

The sulfate and the oxides are important compounds. Natural nickel is a mixture of five stable isotopes; nine other unstable isotopes are known.

Handling:

Exposure to nickel metal and soluble compounds (as Ni) should not exceed 0.05 $mg/cm^3$ (8-hour time-weighted average per 40-hour work week). Nickel sulfide fume and dust is recognized as being potentially carcinogenic.

Lead

History:

(Anglo-Saxon *lead*; L. *plumbum*) Long known, mentioned in Exodus. The alchemists believed lead to be the oldest metal and associated with the planet Saturn. Native lead occurs in nature, but is rare.

Sources:

Lead is obtained chiefly from galena (PbS) by a roasting process. Anglesite, cerussite, and minim are other common lead minerals.

Properties:

Lead is a bluish-white metal of bright luster. It is very soft, highly malleable, ductile, and a poor conductor of electricity. It is very resistant to corrosion; lead pipes bearing the insignia of Roman emperors, used as drains from the baths, are still in service. It is used in containers for corrosive liquids (such as sulfuric acid) and may be toughened by the addition of a small percentage of antimony or other metals.

Forms:

Natural lead is a mixture of four stable isotopes: $^{204}$Pb (1.48%), $^{206}$Pb (23.6%), $^{207}$Pb (22.6%), and $^{208}$Pb (52.3%). Lead isotopes are the end products of each of the three series of naturally occurring radioactive elements: $^{206}$Pb for the uranium series, $^{207}$Pb for the actinium series, and $^{208}$Pb for the thorium series. Twenty-seven other isotopes of lead, all of which are radioactive, are recognized.

Its alloys include solder, type metal, and various antifriction metals. Great quantities of lead, both as the metal and as the dioxide, are used in storage batteries. Much metal also goes into cable covering, plumbing, ammunition, and in the manufacture of lead tetraethyl.

Uses:

The metal is very effective as a sound absorber, is used as a radiation shield around X-ray equipment and nuclear reactors, and is used to absorb vibration. White lead, the basic carbonate, sublimed white lead, chrome yellow, and other lead compounds are used extensively in paints, although in recent years the use of lead in paints has been drastically curtailed to eliminate or reduce health hazards.

Lead oxide is used in producing fine "crystal glass" and "flint glass" of a high index of refraction for achromatic lenses. The nitrate and the acetate are soluble salts. Lead salts such as lead arsenate have been used as insecticides, but their use in recent years has been practically eliminated in favor of less harmful organic compounds.

Handling:

Care must be used in handling lead as it is a cumulative poison. Environmental concerns with lead poisoning have resulted in a national program to eliminate the lead in gasoline.

Zinc

History:

(German *Zink*, of obscure origin) Centuries before zinc was recognized as a distinct element, zinc ores were used for making brass. Tubal-Cain, seven generations from Adam, is mentioned as being an "instructor in every

artificer in brass and iron." An alloy containing 87 percent zinc has been found in prehistoric ruins in Transylvania.

Metallic zinc was produced in the 13th century A.D. India by reducing calamine with organic substances such as wool. The metal was rediscovered in Europe by Marggraf in 1746. He demonstrated that zinc could be obtained by reducing calamine with charcoal.

Sources:

The principal ores of zinc are sphalerite (sulfide), smithsonite (carbonate), calamine (silicate), and franklinite (zinc, manganese, iron oxide). One method of zinc extraction involves roasting its ores to form the oxide and reducing the oxide with coal or carbon, with subsequent distillation of the metal.

Isotopes:

Naturally occurring zinc contains five stable isotopes. Sixteen other unstable isotopes are recognized.

Properties:

Zinc is a bluish-white, lustrous metal. It is brittle at ordinary temperatures but malleable at 100 to 150$^{o}$C. It is a fair conductor of electricity, and burns in air at high red heat with evolution of white clouds of the oxide.

It exhibits superplasticity. Neither zinc nor zirconium is ferromagnetic; but $ZrZn_2$ exhibits ferromagnetism at temperatures below 35$^{o}$K. It has unusual electrical, thermal, optical, and solid-state properties that have not been fully investigated.

Uses:

The metal is employed to form numerous alloys with other metals. Brass, nickel silver, typewriter metal, commercial bronze, spring bronze, German silver, soft solder, and aluminum solder are some of the more important alloys.

Large quantities of zinc are used to produce die-castings, which are used extensively by the automotive, electrical, and hardware industries. An alloy called Prestal (R), consisting of 78 percent zinc and 22 percent aluminum, is reported to be almost as strong as steel and as easy to mold as plastic. The

alloy said to be so moldable that it can be molded into form using inexpensive ceramics or cement die casts.

Zinc is also used extensively to galvanize other metals such as iron to prevent corrosion. Zinc oxide is a unique and very useful material for modern civilization. It is widely used in the manufacture of paints, rubber products, cosmetics, pharmaceuticals, floor coverings, plastics, printing inks, soap, storage batteries, textiles, electrical equipment, and other products. Lithopone, a mixture of zinc sulfide and barium sulfate, is an important pigment. Zinc sulfide is used in making luminous dials, X-ray and TV screens, and fluorescent lights.

The chloride and chromate are also important compounds. Zinc is an essential element in the growth of human beings and animals. Tests show that zinc-deficient animals require 50 percent more food to gain the same weight as an animal supplied with sufficient zinc.

Handling:

Zinc is not considered to be toxic, but when freshly formed ZnO is inhaled a disorder known as *oxide shakes* or *zinc chills* sometimes occurs. Where zinc oxide is encountered, recommendations include providing good ventilation

to avoid concentration exceeding 5 mg/$m^3$, (time-weighted over an 8-hour exposure, 40-hour work week).

Affect on health:

Zinc is an essential element in our diet. Too little zinc can cause problems, but too much zinc is also harmful. Harmful effects generally begin at levels 10-15 times higher than the amount needed for good health. Large doses taken by mouth even for a short time can cause stomach cramps, nausea, and vomiting. Taken longer, it can cause anemia and decrease the levels of your good cholesterol. We do not know if high levels of zinc affect reproduction in humans. Rats that were fed large amounts of zinc became infertile. Inhaling large amounts of zinc (as dusts or fumes) can cause a specific short-term disease called metal fume fever. We do not know the long-term effects of breathing high levels of zinc. Putting low levels of certain zinc compounds on the skin of rabbits, guinea pigs, and mice caused skin irritation. Skin irritation will probably occur in people.

## The Present Study

In the present study, I was interested in learning the cellular location of several heavy metals taken up by cells. I used Al, Cd, Cu, Mn, Ni, Pb, and Zn. In previous data from energy dispersive x-ray (EDAX) studies involving heavy metals, results showed that the majority of the heavy metals were sequestered in the polyphosphate bodies in the microorganisms (Jensen, 1990; Jensen et al., 1982a, Goldberg et al., 2001). Small amounts of the heavy metals were concentrated in the cytoplasm and cell wall.

In order to make the study an encompassing one, I have chosen to use a number of cyanobacteria, gram positive and gram-negative bacteria, in addition to two eukaryotes. The cyanobacteria I have chosen are *Plectonema boryanum* (UTEX 581) (Figure 1 a&b), *Synechococcus leopoliensis* (UTEX B 2434) (Figure 2*)*, and *Gloeocapsa alpicola* (UTEX B 589)(Figure 3); I chose three gram positive bacteria *Bacillus subtilus* Presque Isle collection cat. #620 (Figure 4), *Staphylococcus aureus* Presque Isle collection cat. # +4651 (Figure 5), and *Arthrobacter globiformis* Presque Isle collection cat. # 607 (Figure 6). In addition to these two groups, I chose to examine three-

gram negative bacteria, *Acinetobacter calcoaceticus* Presque Isle collection cat. #346 (Figure 7), and *Pseudomonas aeruginosa,* Presque Isle collection cat. #+99 (Figure 8), and *Escherichia coli* Presque Isle collection cat. # 336 (Figure 9). To make the study an all-encompassing one I also chose two eukaryotic species (yeast), *Saccharomyces cerevisiae,* Presque Isle collection cat. #1015 (Figure 10), and *Rhodotorula rubra* Presque Isle collection cat. # 1010 (Figure 11). All of these organisms contain polyphosphate bodies under the right conditions.

## MATERIALS AND METHODS

In order to make this study more encompassing we used different species of organisms. I chose both prokaryotic and eukaryotic cells. The prokaryotic cells included cyanobacteria, gram-negative bacteria, and gram-positive bacteria. The eukaryotic material consisted of yeasts. All of these were chosen since they are able to make large numbers of polyphosphate bodies.

I also produced cells with many PPBs by using the "overplus phenomenon." Growing cells in a normal manner for the appropriate amount of time and then starving them of a specific element like P or S for a few days and then returning the specific element to the cells does this (Baxter and Jensen, 1986).

The cyanobacteria I chose *Plectonema boryanum* (UTEX 581), *Synechococcus leopoliensis* (UTEX B 2434), and *Gloeocapsa alpicola* (UTEX B 589), all were obtained from the Starr Culture Collection. All the

cultures were grown for two weeks at 21$^{o}$ C in modified Fitzgeralds medium (see appendix A) (Fitzgerald *et al*., 1952; Zender and Gorham, 1960) at pH 7.2. The pH was check using a pH meter. The cells were kept in a Sherer Controlled Environment chamber (Model Cel B) and illuminated with 500 ft candles of cool white fluorescent light, supplemented by two 25W incandescent bulbs. The chamber was set to a 12hour light/dark cycle. All cultures were agitated, by shaking the flasks several times a day.

I have also chosen three-gram positive bacteria, *Bacillus subtilis*, *Staphylococcus aureus*, and *Arthrobacter globiformis*. In addition to these two groups I have chosen to examine three-gram negative bacteria, *Acinetobacter calcoaceticus*, *Escherichia coli* and *Pseudomonas aeruginosa*.

Both the gram negative and gram-positive bacteria were grown for 72 hours in Difco-Bacto nutrient broth. The broth is prepared by adding 8 grams of nutrient broth powder to 1 liter of water. The cells were added by sterile loop to the broth. The cells were incubated in a Fisher Scientific incubator at 37°C. The cells were harvested after the 72 hour period, then rinsed in

distilled water, and then put into distilled water for another 72 hours. This process starves the cells of certain nutrients like P or S. After the second period of 72 hours the cell are again put back into Bacto-Difco nutrient broth. The cells are then left to grow for another 24 hours and then harvested. The cells were then washed three times in distilled water.

I also chose two eukaryotes (yeast), *Saccharomyces cerevisiae*, and *Rhodotorula rubra*. Each of the yeasts was grown in Sabouraud broth for 72 hours in Difco-Bacto Sabouraud broth. The broth is prepared by adding 65 grams of Sabouraud broth powder to 1000 ml of water. The solution is then spun with a stir bar. The solution is then autoclaved for 15 minutes at 15 pounds of pressure. The cells were added by sterile loop to the broth. The cells were incubated in a Fisher Scientific incubator at 37°c. The cells were harvested after the 72 hour period, then rinsed in distilled water, and then put into distilled water for another 72 hours. This process starves the cells of certain nutrients like P or S. After the second period of 72 hours the cell are again put back into nutrient agar by Bacto-Difco. The cells are then left to grow for another 24 hours and then harvested. The cells were then washed three times in distilled water.

Exposure to Heavy Metals

The cells were then exposed to seven heavy metals, independently, those being Al, Cd, Cu, Mn, Ni, Pb, and Zn. Exposure was done at one concentration, 20 parts per million (PPM), except for Cd which is very toxic and I used only 5 PPM. For every organism I also used a set of controls for each cell type and metal exposure. The cells were exposed to each metal for a total of ninety minutes. The cyanobacterial cells were kept in the Sherer Controlled Environment chamber (Model Cel B) and illuminated with 500 ft candles of cool white fluorescent light, supplemented by two 25W incandescent bulbs, for the allotted amount of time. In addition the cells were kept under constant agitation on a platform stirrer.

The gram negative, gram positive and yeast cells were all kept in the 37° incubator for the ninety-minute span of time. These cells were also kept under constant agitation in the incubator. The cells were removed from the individual heavy metal, washed three times and prepared for observation and analysis under the electron microscope in two ways.

All cells were tested for their viability, after their exposure to the heavy metal solution. A small sample of each of the exposed cells was put back into its respective growth media, in order to determine if indeed the cells would survive exposure to the metal. In each case the cells grew in their assigned media, showing that the exposure to the metal was not toxic.

Electron Microscopy

Cells were fixed in 1% osmium tetraoxide (see Appendix B) as described by Kellenberger et al. (1958) and modified by Pankratz and Bowen (1963). After fixation cells were dehydrated in ethanol followed by propylene oxide then embedded in Epon (Luft, 1961) (see appendix c). Sections were cut using a diamond knife and were collected on either copper or nickel grids, depending on which metal the cells were exposed to. The cells were then post stained with uranyl acetate (Stempak and Ward, 1964) (see appendix d). The cells were then examined with a Hitachi H-7000 transmission electron microscope (TEM) at 75kV.

Air-dried cells

It was demonstrated by Baxter and Jensen (1980a), that the air-drying method is simple and efficient; most importantly, it is a method that can prevent serious elemental changes in the preparation process. It is therefore frequently used to prepare cells for EDAX.

The cells were air dried on a formvar-coated grid and air dried at room temperature the cells after being totally dried were ready to be studied. I analyzed twenty cells in each treatment and in each control.

The samples were then located and photographed using a Hitachi-7000 TEM in stem mode in conjunction with a PGT Integrated Microanalyzer and Imaging and X-ray (IMIX) system.

With the microscope in STEM (spot mode), x-ray collections were made for 100 seconds with the probe placed on either the polyphosphate body, cell wall, sheath, or their cytoplasm. These readings were subtracted from a

background spectrum of either the formvar or the Epon in order to be able to receive a true unadulterated answer.

The results are two fold, I first get the qualitative analysis data, which reveals the elemental content of the cells, and then I perform quantitative analysis (Figures 13-14). This gives me the percentages of the samples by using the IMIX system's bulk sample quantitative analysis module in the ZAF correction method. These percentages correspond to the ppm amounts of each element including the heavy metal, found in the cell. Table 2 is a summary of the data received from the quantitative analysis performed on the cells.

Given the percentages of the elements contained in the samples, the approximate amounts of the metals contained in the different components of the cells, is calculated by measuring the diameter of the cell and calculating its volume. The volumes of the cells are calculated by determination of the shape of the cell and then using the classic formula in order to determine the volume. The formulas used in order to determine the volumes are: for a

cylindrically shaped organism "pi x radius$^2$ x height". For organisms that have the shape of a sphere I used the formula "(4/3) x pi x radius$^{3"}$. In order to determine the height or the radius of the organism I measured the components of the organism on a micrograph using a ruler. The original units were millimeters and then converted into micrometers. I measured twenty cells per treatment, and then took the mean of all the cells, in that particular treatment. The mean volumes measured in $\mu m^3$ of the different cells are shown in Table 1.

I then used Hall's theories, to calculate the mass of the specimen. Calculating the continuum (background noise) in x-ray microanalyzed spectra was previously shown by Dr. Hall et al. (1974). To be related to the complex interelemental effects. This gives you the quantitative amounts of the respective metals found in the different parts of the cells. There was very little variation in the quantitative analysis done on each individual cell for each cell type. Taking this into consideration the means for the twenty cells were calculated using Statview. These calculations are shown in Table 2.

I in turn determined which cell area or inclusion has sequestered a metal and then how much. We can then extrapolate these data to the whole cell. I calculated the volume of the capsule, cell wall, cytoplasm, and the PPBs. When I used the data obtained from the quantitative analysis and applied the numbers from the analysis to the volumes of the particular components I was able to determine the mean amounts in ppm/$\mu m^3$ present in the individual cell components (Tables 3-13).

In addition all results from each of the twenty cells tested and their respective inclusions/organelles were analyzed using the statistical programs, those of Statview and JMP (SAS). This gave us the data we need to determine which cell component sequesters a metal in the largest amount (Baxter and Jensen 1986). These numbers can be found in Appendix E, and are represented by the bar graphs. Each mean is graphed with ± 1 SE. The cell mean is representative of the parts per million (PPM) of the metal found in the twenty cells and their respective cell inclusions/organelles analyzed.

| Table 1 Volumes of respective cellular componenets | Volume Capsule | Volume Cell Wall | Volume Cytoplasm | Volume PPB |
|---|---|---|---|---|
| **All numbers are in micrometers cubed** | | | | |
| ***Plectonema boryanum*** | **1.67µm$^3$** | **1.3 µm$^3$** | **86.9 µm$^3$** | **38.7 µm$^3$** |
| ***Synechoccus leopoliensis*** | | **1.05 µm$^3$** | **91 µm$^3$** | **25.4 µm$^3$** |
| ***Gloeocapsa alpicola*** | **1.69 µm$^3$** | **1.4 µm$^3$** | **91.8 µm$^3$** | **31.4 µm$^3$** |
| ***Staphylococcus aureus*** | **1.003 µm$^3$** | **0.85 µm$^3$** | **14.61 µm$^3$** | **5.96 µm$^3$** |
| ***Bacillus subtilus*** | **1.09 µm$^3$** | **0.732 µm$^3$** | **13.79 µm$^3$** | **4.61 µm$^3$** |
| ***Arthrobacter globiformis*** | **1.1 µm$^3$** | **0.698 µm$^3$** | **18.45µm$^3$** | **3.89 µm$^3$** |
| ***Acinetobacter calcoaceticus*** | **1.074 µm$^3$** | **0.96 µm$^3$** | **10.71µm$^3$** | **6.79 µm$^3$** |
| ***Pseudomonas aeruginosa*** | **1.03 µm$^3$** | **0.893 µm$^3$** | **16.27 µm$^3$** | **5.13 µm$^3$** |
| ***Escherichia coli*** | **1.053 µm$^3$** | **0.97 µm$^3$** | **16.04µm$^3$** | **4.27 µm$^3$** |
| ***Saccharomyces cerevisiae*** | **1.12 µm$^3$** | **0.99 µm$^3$** | **19.34µm$^3$** | **5.96 µm$^3$** |
| ***Rhodotorula rubra*** | **1.2 µm$^3$** | **1.003 µm$^3$** | **17.66 µm$^3$** | **6.34 µm$^3$** |

**Table 1: Volumes of the various cellular components.**

**Table 2: Mean amounts of metals found in cells. Units are parts per million**

| ***Plectonema Boryanum*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
|---|---|---|---|---|---|---|---|
| **Capsule** | **7.49** | **8.517** | **7.418** | **6.625** | **7.278** | **7.149** | **5.821** |
| **Cell Wall** | **7.154** | **10.655** | **5.492** | **7.313** | **7.335** | **7.693** | **6.755** |
| **Cytoplasm** | **9.041** | **9.118** | **5.819** | **9.087** | **7.227** | **6.086** | **5.904** |
| **PPB** | **13.636** | **16.221** | **11.972** | **15.355** | **12.3** | **16.852** | **11.249** |
| | | | | | | | |
| ***Synechococcus leopolienisis*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Cell Wall** | **8.074** | **10.659** | **7.125** | **6.417** | **10.001** | **9.037** | **7.15** |
| **Cytoplasm** | **7.31** | **12.724** | **8.454** | **7.802** | **7.256** | **7.418** | **9.156** |
| **PPB** | **14.973** | **14.061** | **12.041** | **13.029** | **11.166** | **10.698** | **12.322** |
| | | | | | | | |
| ***Gloeocapsa alpicola*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **5.548** | **5.799** | **7.537** | **6.03** | **5.991** | **5.796** | **8.487** |
| **Cell Wall** | **8.057** | **6.319** | **7.655** | **8.007** | **7.297** | **5.434** | **6.642** |
| **Cytoplasm** | **7.043** | **6.145** | **8.173** | **6.403** | **7.951** | **8.059** | **7.664** |
| **PPB** | **12.176** | **7.494** | **10.789** | **14.45** | **10.244** | **12.484** | **12.082** |
| | | | | | | | |
| ***Bacillus subtilis*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **6.99** | **5.848** | **5.796** | **6.695** | **6.908** | **6.587** | **6.78** |
| **Cell Wall** | **8.05** | **12.255** | **5.771** | **5.821** | **6.034** | **7.042** | **7.904** |
| **Cytoplasm** | **6.065** | **9.964** | **6.486** | **6.948** | **7.549** | **5.675** | **6.774** |
| **PPB** | **12.176** | **12.765** | **12.748** | **13.941** | **12.511** | **15.693** | **14.774** |
| | | | | | | | |
| **Staphylococcus aureus** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **6.49** | **10.017** | **7.418** | **5.79** | **7.278** | **6.535** | **6.993** |
| **Cell Wall** | **6.654** | **11.655** | **5.492** | **7.073** | **7.335** | **7.843** | **6.084** |
| **Cytoplasm** | **7.541** | **10.018** | **5.819** | **7.237** | **7.227** | **7.237** | **7.252** |
| **PPB** | **13.636** | **11.221** | **11.972** | **14.926** | **12.3** | **14.253** | **15.185** |
| | | | | | | | |
| ***Arthrobacter globiformis*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **6.99** | **7.435** | **6.695** | **6.378** | **4.918** | **5.69** | **6.934** |
| **Cell Wall** | **6.964** | **10.669** | **5.633** | **7.024** | **5.921** | **6.157** | **7.001** |
| **Cytoplasm** | **6.641** | **10.271** | **6.758** | **6.858** | **7.216** | **6.76** | **7.252** |
| **PPB** | **14.973** | **12.258** | **14.845** | **14.879** | **14.998** | **14.948** | **20.03** |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***E.coli*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **6.477** | **10.081** | **5.609** | **7.311** | **7.189** | **6.605** | **7.28** |
| **Cell Wall** | **9.041** | **10.618** | **5.492** | **7.364** | **7.227** | **7.371** | **7.258** |
| **Cytoplasm** | **7.322** | **8.063** | **7.313** | **6.226** | **7.009** | **6.979** | **6.218** |
| **PPB** | **6.477** | **10.081** | **5.609** | **7.311** | **7.189** | **6.605** | **7.28** |
| | | | | | | | |
| ***Acinetobacter calcoaceticus*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **7.011** | **11.434** | **6.349** | **7.043** | **9.017** | **6.654** | **6.851** |
| **Cell Wall** | **6.559** | **11.042** | **6.061** | **10.875** | **6.347** | **7.461** | **6.059** |
| **Cytoplasm** | **7.322** | **8.063** | **7.313** | **6.226** | **7.009** | **6.979** | **6.218** |
| **PPB** | **14.598** | **14.5** | **13.167** | **14.799** | **14.917** | **13.339** | **14.708** |
| | | | | | | | |
| ***Pseudomonas aeruginosa*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **6.669** | **11.637** | **6.444** | **7.012** | **9.095** | **6.244** | **7.054** |
| **Cell Wall** | **6.477** | **10.081** | **5.609** | **7.311** | **7.189** | **6.605** | **7.28** |
| **Cytoplasm** | **9.304** | **12.312** | **7.323** | **6.047** | **9.33** | **8.771** | **7.072** |
| **PPB** | **13.636** | **16.221** | **11.972** | **15.355** | **12.3** | **16.852** | **11.249** |
| | | | | | | | |
| ***Saccharomyces cerevisiae*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **5.641** | **10.017** | **7.418** | **5.79** | **7.278** | **6.535** | **6.993** |
| **Cell Wall** | **6.353** | **11.655** | **5.492** | **7.073** | **7.335** | **7.843** | **6.804** |
| **Cytoplasm** | **7.541** | **10.018** | **5.819** | **7.237** | **7.727** | **7.163** | **7.252** |
| **PPB** | **13.636** | **11.221** | **11.972** | **14.926** | **12.3** | **14.253** | **15.185** |
| | | | | | | | |
| ***Rhodotorula rubra*** | **Al** | **Cd** | **Cu** | **Pb** | **Mn** | **Ni** | **Zn** |
| **Capsule** | **6.154** | **10.655** | **7.355** | **6.535** | **7.244** | **6.511** | **6.695** |
| **Cell Wall** | **7.49** | **8.517** | **7.244** | **6.56** | **7.335** | **7.228** | **5.597** |
| **Cytoplasm** | **9.041** | **10.618** | **5.492** | **7.364** | **7.227** | **7.371** | **7.258** |
| **PPB** | **13.636** | **11.221** | **11.972** | **16.072** | **12.3** | **22.18** | **15.28** |

**Table 2: Mean amounts of metals found in cells. Units are parts per million.**

| *P.boryanum* | | | | |
|---|---|---|---|---|
| | **Capsule** | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.119µm$^3$** | **0.097 µm$^3$** | **11.30 µm$^3$** | **15.8 µm$^3$** |
| **Cd** | **0.177µm$^3$** | **0.11µm$^3$** | **14.4µm$^3$** | **18.42µm$^3$** |
| **Cu** | **0.096µm$^3$** | **0.092µm$^3$** | **7.31µm$^3$** | **9.26 µm$^3$** |
| **Mn** | **0.129µm$^3$** | **0.094µm$^3$** | **9.077µm$^3$** | **9.52µm$^3$** |
| **Ni** | **0.119µm$^3$** | **0.109µm$^3$** | **5.288µm$^3$** | **6.3µm$^3$** |
| **Pb** | **0.1100µm$^3$** | **0.095µm$^3$** | **7.896µm$^3$** | **11.88µm$^3$** |
| **Zn** | **0.0972µm$^3$** | **0.088µm$^3$** | **5.130µm$^3$** | **8.706µm$^3$** |

**Table 3: Amounts of metals in individual components of *P.boryanum* reported in µm$^3$.**

| *Synechoccus leopoliensis* | | | |
|---|---|---|---|
| | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.084µm$^3$** | **6.65µm$^3$** | **11.40µm$^3$** |
| **Cd** | **0.112µm$^3$** | **11.57µm$^3$** | **10.71µm$^3$** |
| **Cu** | **0.0748µm$^3$** | **7.69µm$^3$** | **9.17µm$^3$** |
| **Mn** | **0.105µm$^3$** | **6.60 µm$^3$** | **8.50µm$^3$** |
| **Ni** | **0.094µm$^3$** | **6.75µm$^3$** | **8.15µm$^3$** |
| **Pb** | **0.067µm$^3$** | **7.09µm$^3$** | **9.92µm$^3$** |
| **Zn** | **0.075µm$^3$** | **8.33µm$^3$** | **9.38µm$^3$** |

**Table 4: Amounts of metals in individual components of *Synechoccus leopoliensis* reported in µm$^3$.**

| *Gloeocapsa alpicola* | | | | |
|---|---|---|---|---|
| | **Capsule** | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.093µm$^3$** | **0.113µm$^3$** | **6.47µm$^3$** | **7.64 µm$^3$** |
| **Cd** | **0.336µm$^3$** | **0.088µm$^3$** | **5.64µm$^3$** | **4.71µm$^3$** |
| **Cu** | **0.127µm$^3$** | **0.107µm$^3$** | **7.50µm$^3$** | **8.56µm$^3$** |
| **Mn** | **0.101µm$^3$** | **0.102µm$^3$** | **7.29µm$^3$** | **9.65µm$^3$** |
| **Ni** | **0.097µm$^3$** | **0.076µm$^3$** | **7.39µm$^3$** | **7.83µm$^3$** |
| **Pb** | **0.102µm$^3$** | **0.112µm$^3$** | **5.87µm$^3$** | **9.07µm$^3$** |
| **Zn** | **0.143µm$^3$** | **0.092µm$^3$** | **7.03µm$^3$** | **3.79µm$^3$** |

**Table 5: Amounts of metals in individual components of *Gloeocapsa alpicola* reported in µm$^3$.**

| *Bacillus subtilis* | | | | |
|---|---|---|---|---|
| | **Capsule** | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.076µm$^3$** | **0.058µm$^3$** | **0.836µm$^3$** | **1.12µm$^3$** |
| **Cd** | **0.063µm$^3$** | **0.089µm$^3$** | **1.37µm$^3$** | **1.17µm$^3$** |
| **Cu** | **0.063µm$^3$** | **0.042µm$^3$** | **0.894µm$^3$** | **1.17µm$^3$** |
| **Mn** | **0.069µm$^3$** | **0.048µm$^3$** | **0.622µm$^3$** | **1.31 µm$^3$** |
| **Ni** | **0.072µm$^3$** | **0.0515µm$^3$** | **0.782µm$^3$** | **1.44µm$^3$** |
| **Pb** | **0.072µm$^3$** | **0.042µm$^3$** | **0.958µm$^3$** | **1.28µm$^3$** |
| **Zn** | **0.073µm$^3$** | **0.057µm$^3$** | **0.934µm$^3$** | **1.36µm$^3$** |

**Table 6: Amounts of metals in individual components of *Bacillus subtilis* reported in µm$^3$.**

| *Staphylococcus aureus* | | | | |
|---|---|---|---|---|
| | **Capsule** | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.065µm$^3$** | **0.056µm$^3$** | **0.056µm$^3$** | **0.065µm$^3$** |
| **Cd** | **0.80µm$^3$** | **0.090µm$^3$** | **1.46µm$^3$** | **1.33µm$^3$** |
| **Cu** | **0.125µm$^3$** | **0.046µm$^3$** | **0.850µm$^3$** | **1.42µm$^3$** |
| **Mn** | **0.075µm$^3$** | **0.062µm$^3$** | **1.055µm$^3$** | **1.46µm$^3$** |
| **Ni** | **0.066µm$^3$** | **0.065µm$^3$** | **1.05µm$^3$** | **1.69µm$^3$** |
| **Pb** | **0.058µm$^3$** | **0.060µm$^3$** | **1.087µm$^3$** | **1.77µm$^3$** |
| **Zn** | **0.070µm$^3$** | **0.05µm$^3$** | **1.05µm$^3$** | **1.81µm$^3$** |

**Table 7: Amounts of metals in individual components of *Staphylococcus aureus* reported in µm$^3$.**

| *Arthrobacter globiformis* | | | | |
|---|---|---|---|---|
| | **Capsule** | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.076µm$^3$** | **0.048µm$^3$** | **1.225µm$^3$** | **1.75µm$^3$** |
| **Cd** | **0.081µm$^3$** | **0.074µm$^3$** | **1.89µm$^3$** | **1.43µm$^3$** |
| **Cu** | **0.073µm$^3$** | **0.0393µm$^3$** | **1.24µm$^3$** | **1.73µm$^3$** |
| **Mn** | **0.055µm$^3$** | **0.041µm$^3$** | **1.33 µm$^3$** | **1.75µm$^3$** |
| **Ni** | **0.063µm$^3$** | **0.043µm$^3$** | **1.23µm$^3$** | **1.74µm$^3$** |
| **Pb** | **0.070µm$^3$** | **0.49 µm$^3$** | **1.265µm$^3$** | **1.736µm$^3$** |
| **Zn** | **0.076µm$^3$** | **0.048µm$^3$** | **1.348µm$^3$** | **1.558µm$^3$** |

**Table 8 Amounts of metals in individual components of *Arthrobacter globiformis* reported in µm$^3$.**

| *Acinetobacter calcoaceticus* | | | | |
|---|---|---|---|---|
| | **Capsule** | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.075µm$^3$** | **0.629µm$^3$** | **0.784µm$^3$** | **0.991µm$^3$** |
| **Cd** | **0.122µm$^3$** | **0.106µm$^3$** | **0.863µm$^3$** | **0.984µm$^3$** |
| **Cu** | **0.068µm$^3$** | **0.058µm$^3$** | **0.783µm$^3$** | **0.894µm$^3$** |
| **Mn** | **0.096µm$^3$** | **0.060µm$^3$** | **0.750µm$^3$** | **1.012µm$^3$** |
| **Ni** | **0.0714µm$^3$** | **0.0716µm$^3$** | **0.747µm$^3$** | **0.950µm$^3$** |
| **Pb** | **0.0756µm$^3$** | **0.0702µm$^3$** | **0.666µm$^3$** | **1.004µm$^3$** |
| **Zn** | **0.0735µm$^3$** | **0.0581µm$^3$** | **0.665µm$^3$** | **0.998µm$^3$** |

**Table 9: Amounts of metals in individual components of *Acinetobacter calcoaceticus* reported in µm$^3$.**

| *Pseudomonas aeruginosa* | | | | |
|---|---|---|---|---|
| | **Capsule** | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.686µm$^3$** | **0.0578µm$^3$** | **1.513µm$^3$** | **2.098 µm$^3$** |
| **Cd** | **0119µm$^3$** | **0.090 µm$^3$** | **2.003µm$^3$** | **0.987µm$^3$** |
| **Cu** | **0.066µm$^3$** | **0.050µm$^3$** | **1.191µm$^3$** | **1.22µm$^3$** |
| **Mn** | **0.083µm$^3$** | **0.064µm$^3$** | **1.51µm$^3$** | **1.81µm$^3$** |
| **Ni** | **0.0643µm$^3$** | **0.0634µm$^3$** | **1.457µm$^3$** | **1.457µm$^3$** |
| **Pb** | **0.722µm$^3$** | **0.0.065µm$^3$** | **0.983µm$^3$** | **1.577µm$^3$** |
| **Zn** | **0.072µm$^3$** | **0.065µm$^3$** | **1.151µm$^3$** | **1.154µm$^3$** |

**Table 10: Amounts of metals in individual components of *Pseudomonas aeruginosa* reported in µm$^3$.**

| *E. coli* | | | | |
|---|---|---|---|---|
| | **Capsule** | **Cell wall** | **Cytoplasm** | **PPB** |
| **Al** | **0.08µm$^3$** | **0.054µm$^3$** | **1.40µm$^3$** | **1.66µm$^3$** |
| **Cd** | **0.725µm$^3$** | **0.101 µm$^3$** | **1.601µm$^3$** | **1.04µm$^3$** |
| **Cu** | **0.060µm$^3$** | **0.062µm$^3$** | **0.95µm$^3$** | **1.022µm$^3$** |
| **Mn** | **0.076µm$^3$** | **0.0711µm$^3$** | **1.1592µm$^3$** | **1.57µm$^3$** |
| **Ni** | **0.068µm$^3$** | **0.06 µm$^3$** | **1.102µm$^3$** | **1.28µm$^3$** |
| **Pb** | **0.066µm$^3$** | **0.063µm$^3$** | **1.18µm$^3$** | **1.37µm$^3$** |
| **Zn** | **0.069µm$^3$** | **0.064µm$^3$** | **1.164µm$^3$** | **1.3µm$^3$** |

**Table 11: Amounts of metals in individual components of *E. coli* reported in µm$^3$.**

| ***Saccharomyces cerevisiae*** | | | | |
|---|---|---|---|---|
| | Capsule | Cell wall | **Cytoplasm** | **PPB** |
| **Al** | **0.063µm$^3$** | **0.062µm$^3$** | **1.16µm$^3$** | **1.625µm$^3$** |
| **Cd** | **0.112µm$^3$** | **0.115µm$^3$** | **1.93µm$^3$** | **1.33µm$^3$** |
| **Cu** | **0.083µm$^3$** | **0.054µm$^3$** | **1.12µm$^3$** | **1.42µm$^3$** |
| **Mn** | **0.082µm$^3$** | **0.072µm$^3$** | **1.49µm$^3$** | **1.54µm$^3$** |
| **Ni** | **0.073µm$^3$** | **0.077µm$^3$** | **1.38µm$^3$** | **1.698µm$^3$** |
| **Pb** | **0.064µm$^3$** | **0.07µm$^3$** | **1.39 µm$^3$** | **1.77µm$^3$** |
| **Zn** | **0.078µm$^3$** | **0.067µm$^3$** | **1.40µm$^3$** | **1.81µm$^3$** |

**Table 12: Amounts of metals in individual components of *Saccharomyces cerevisiae* reported in µm$^3$.**

| ***Rhodotorula rubra*** | | | | |
|---|---|---|---|---|
| | Capsule | Cell wall | **Cytoplasm** | **PPB** |
| **Al** | **0.089µm$^3$** | **0.061µm$^3$** | **1.59µm$^3$** | **1.73µm$^3$** |
| **Cd** | **0.102µm$^3$** | **0.106 µm$^3$** | **1.69µm$^3$** | **1.48µm$^3$** |
| **Cu** | **0.09µm$^3$** | **0.055µm$^3$** | **1.027µm$^3$** | **1.51µm$^3$** |
| **Mn** | **0.86 µm$^3$** | **0.735µm$^3$** | **1.27µm$^3$** | **1.56µm$^3$** |
| **Ni** | **0.078µm$^3$** | **0.072µm$^3$** | **1.29µm$^3$** | **1.40µm$^3$** |
| **Pb** | **0.078µm$^3$** | **0.065µm$^3$** | **1.424µm$^3$** | **2.03µm$^3$** |
| **Zn** | **0.08µm$^3$** | **0.056 µm$^3$** | **1.28µm$^3$** | **1.93µm$^3$** |

**Table 13: Amounts of metals in individual components of *Rhodotorula rubra* reported in µm$^3$.**

# RESULTS AND OBSERVATIONS

## ***Cyanobacteria***

### Plectonema boryanum

Figure 1a shows a typical image of an air-dried cell of *Plectonema boryanum* and Figure 1b is a micrograph taken of a thin sectioned *Plectonema boryanum*, the electron dense bodies in the cells are the polyphosphate bodies. Measurment of the PPBs at 20,000 x magnification showed that they average 4.2μm in diameter. The capsule averages 0.023 μm in size and the cell wall averages 0.01.5 μm in size. Measurement of the cytoplasm gives an average of 18.6 μm in size.

The cells of *Plectonema boryanum* that were exposed to seven heavy metals independently, showed ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPBs) were analyzed using x-ray analysis. For each heavy metal that the cells were exposed to, twenty random cells were

analyzed. In addition a control group of *Plectonema boryanum* cells was also analyzed. Figure 12 is an example of a typical x-ray or EDAX spectrum of the wall of a *P.boryanum* cell exposed to Mn. It shows the presence of C, O, a range of major elements that are characteristic of this species. Mn is also evident by the peak in the spectrum. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. In each case the control showed no trace of the test metal.

I report here only the amounts of the particular heavy metal that the cells were exposed to. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 2, please keep in mind that the results are reported in percentages, which according to Ted Juzwac, Jr, the manager of the microanalysis applications lab of the Princeton Gamma Tech (PGT) corporation, 1200 State Road, Princeton New Jersey 08540, who are the makers of this unit and its software; parts per million or ppm are equivalent to the values under the column header “Norm Wt.%”(fig.13-14).

The results from the quantitative analysis of the different cellular components i.e. capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of the Statview 4 and JMP 4. The results from the Statview program are represented here by the bar plots in Figures 15-21 and the results of the one-way ANOVA. The JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 3.This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

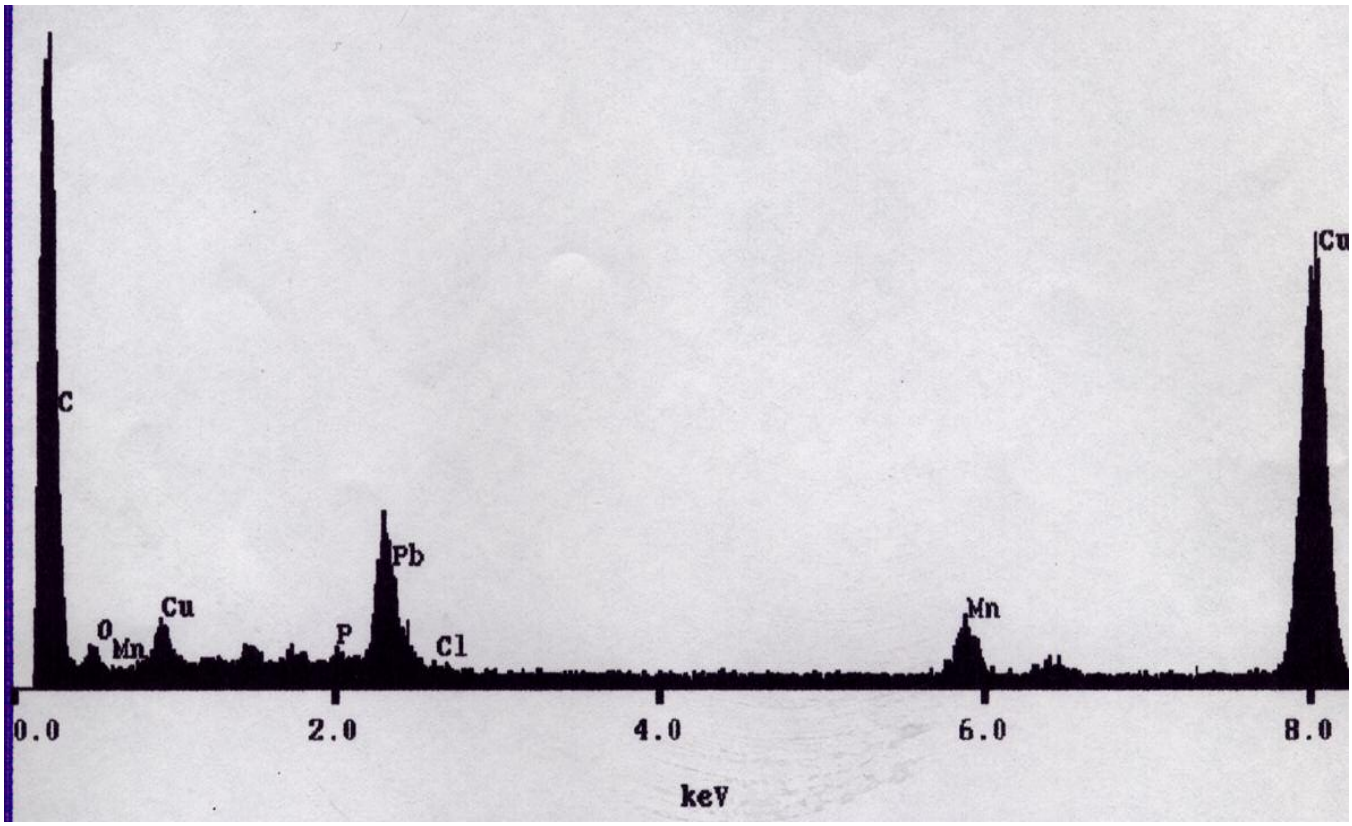



**Figure 12. Spectrum of a cell wall of an air-dried cell of *P.boryanum*. Exposed to 20 ppm of Mn.**

Accelerating Voltage: 75.00 keV
Takeoff Angle: 68.00 degrees
Library for system standards: /imix/spectra/system_standards.dir

| Elm | Rel. K | ZAF | Norm wt% | Prec. | Standard |
|---|---|---|---|---|---|
| C | 0.0727 | 7.6062 | 55.32 | 2.92 | (S)C_K |
| O | 0.0125 | 25.5392 | 31.88 | 10.32 | (S)O_K |
| P | 0.0075 | 1.4757 | 1.11 | 0.13 | (S)P_K |
| Pb | 0.0451 | 0.6547 | 2.95 | 6.03 | (S)Pb_M |
| Cl | 0.0655 | 1.3341 | 8.74 | 1.52 | (S)Cl_K |
| Total | | | 100.00 | | |

Accelerating Voltage: 75.00 keV
Takeoff Angle: 68.00 degrees
Library for system standards: /imix/spectra/system_standards.dir

| Elm | Rel. K | ZAF | Norm wt% | Prec. | Standard |
|---|---|---|---|---|---|
| C | 0.0000 | 0.0000 | 25.44 | 0.00 | |
| O | 0.0587 | 10.1134 | 59.34 | 19.21 | (S)O_K |
| P | 0.0308 | 1.9447 | 5.99 | 0.69 | (S)P_K |
| Pb | 0.0961 | 0.9602 | 9.23 | 18.88 | (S)Pb_M |
| Total | | | 100.00 | | |

**Figure 13: Example of a readout of quantitative analysis "Norm wt%" is compared to PPM.**

**Figure 14: Example of a readout of quantitative analysis "Norm wt%" is compared to PPM.**

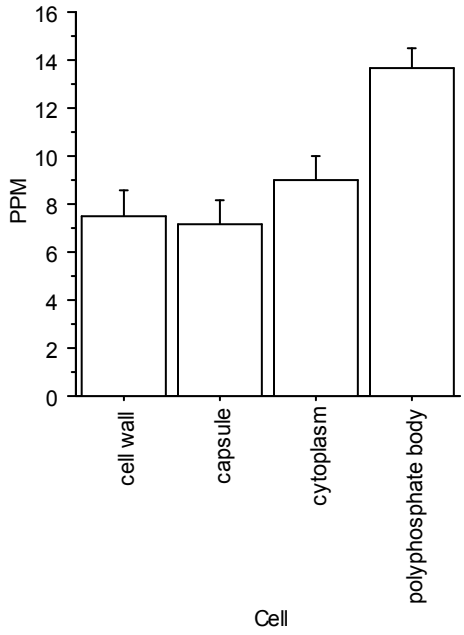



Figure 15: Bar graph for *Plectonema boryanum* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA ($F=9.389$; 3,xdf: $p<0.0001$).

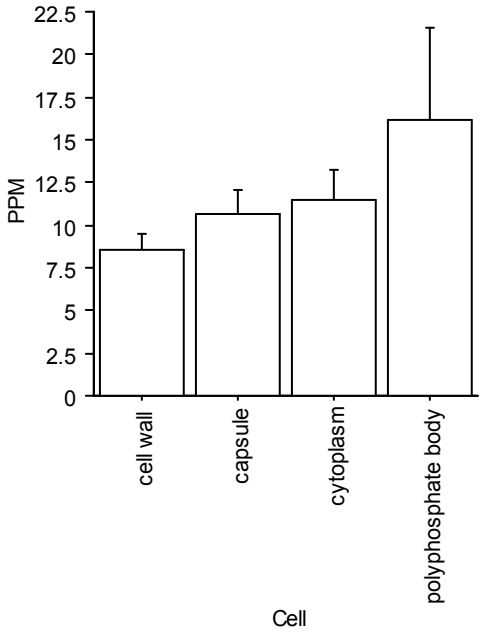



Figure 16: Bar graph for *Plectonema boryanum* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA (F=1.231; 3,xdf: p=0.3044).

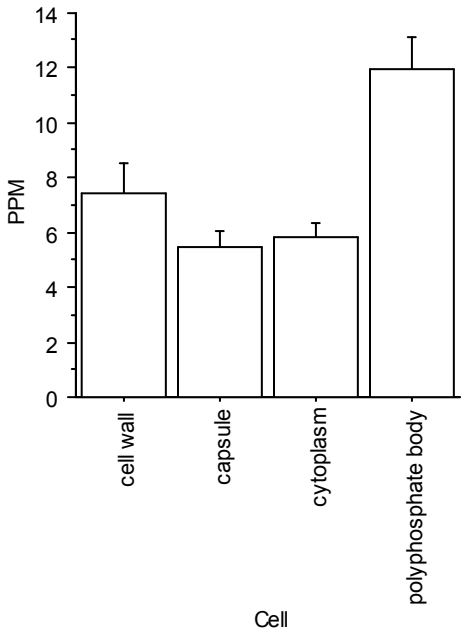



Figure 17: Bar graph for *Plectonema boryanum* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA ($F=11.218$; 3,xdf: $p<0.0001$).

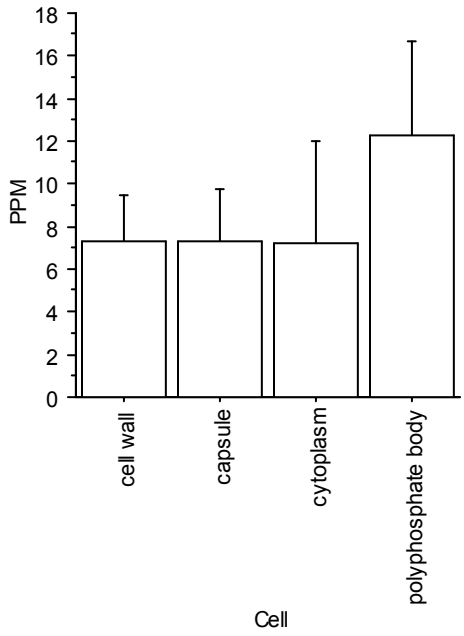



Figure 18: Bar graph for *Plectonema boryanum* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA ($F=9.642$; 3,xdf: $p<0.0001$).

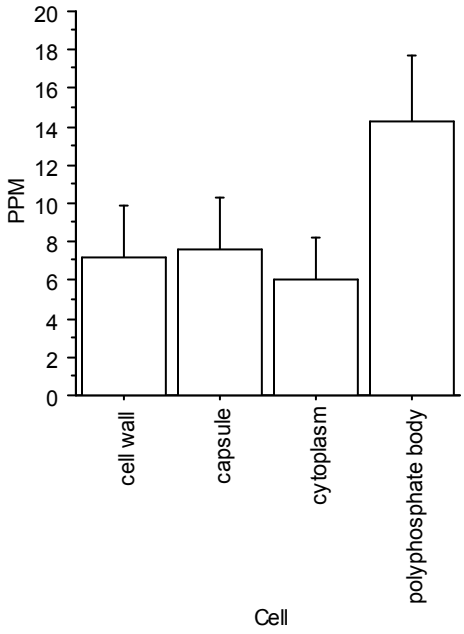



Figure 19: Bar graph for *Plectonema boryanum* exposed to Ni. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA ($F=36.915$; 3,xdf: $p<0.0001$).

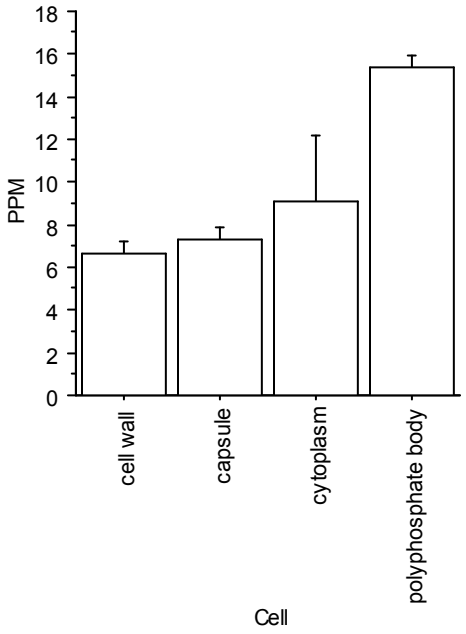



Figure 20: Bar graph for *Plectonema boryanum* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA (F=6.048; 3,xdf: p=0.0009).

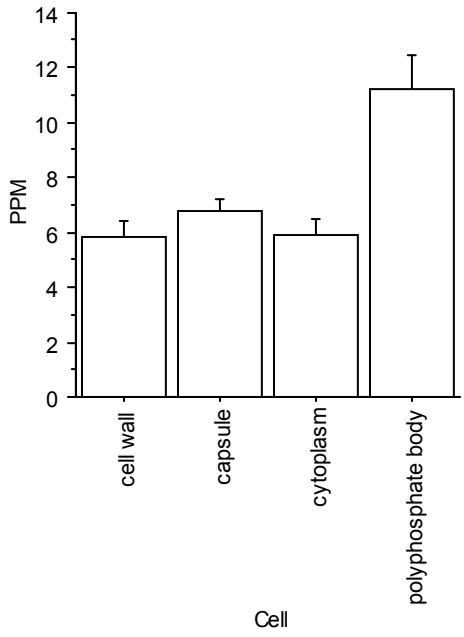



Figure 21: Bar graph for *Plectonema boryanum* exposed to Zn. The numbers for the cell means are given in PPM's. ±1SE the means are significant in ANOVA ($F=11.295$; 3,xdf: $p<0.0001$).

*Synechoccoccus leopoliensis*

The cells of *Synechoccoccus leopoliensis* that were exposed to seven heavy metals independently of each other, showed ability to uptake metal in different areas or inclusions of the cell. The three main areas, cell wall, cytoplasm and polyphosphate bodies (PPBs) were analyzed using x-ray analysis. The *Synechoccoccus leopoliensis* did not show any signs of having a capsule. For each heavy metal that the cells were exposed to twenty random cells were analyzed. In addition a control group of *Synechoccoccus leopoliensis* cells was also analyzed. Figure 22 is an example of a typical x-ray or EDAX spectrum of the polyphosphate body of a *Synechoccoccus leopoliensis* cell exposed to 20 ppm of Al. It shows the presence of C, O, S, K, and of course P representative of the polyphosphate body. Al is also evident by the peak in the spectrum. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. In each case the control showed no trace of the test metal.

I report here only the amounts of the particular heavy metal to which the cells were exposed. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 13; parts per million or ppm are equivalent to the values under the column header "Norm Wt.%"(fig.13-14).

The results from the quantitative analysis of the different cellular components i.e. capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of the Statview 4 and JMP 4. The results from Statview are represented here by the bar plots in Figures 23-29 and the results of the one-way ANOVA. The JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 4. This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

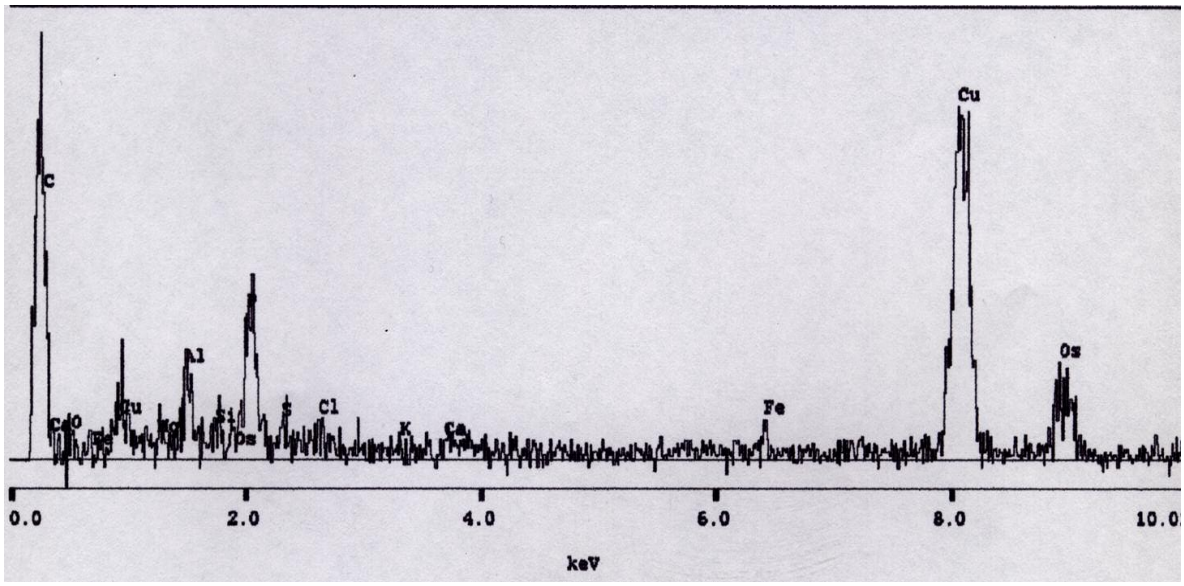



Figure 22. Spectrum of a polyphosphate body of a sectioned cell of *Synechoccoccus leopoliensis* exposed to 20 ppm of aluminum.

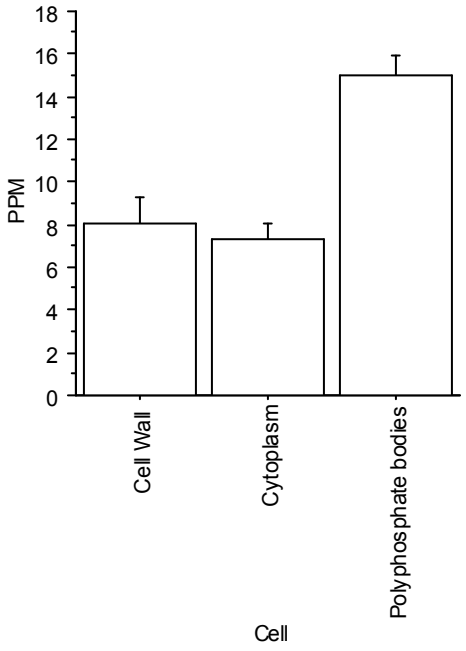



Figure 23: Bar graph for *Synechoccoccus leopoliensis* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA ($F=19.593$; 3,xdf: $p<0.0001$).

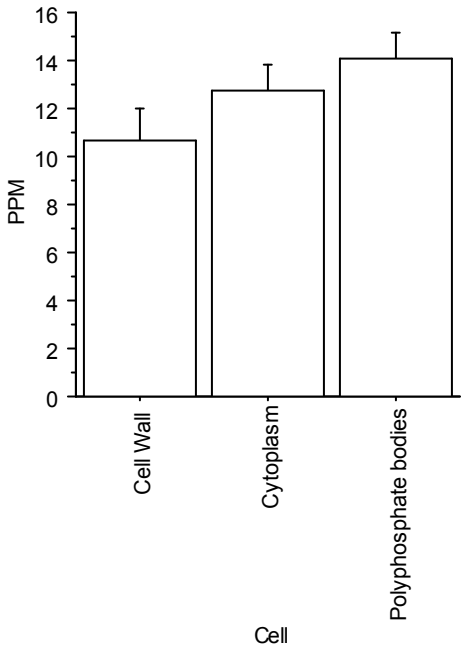



Figure 24: Bar graph for *Synechoccoccus leopoliensis* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA (F=1.998; 3,xdf: p=0.1450).

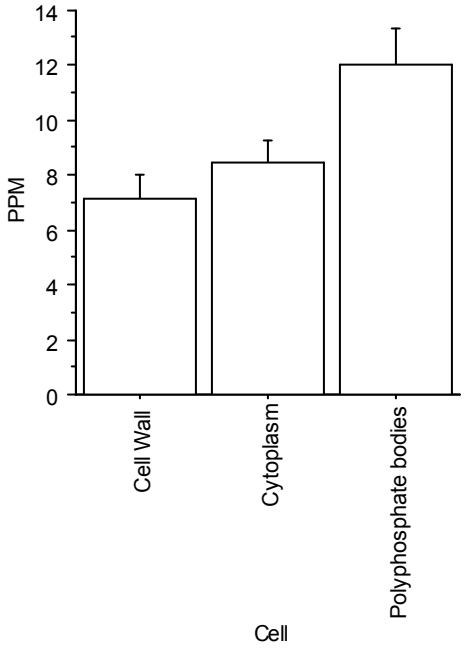



Figure 25: Bar graph for *Synechoccoccus leopoliensis* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA (F=6.345; 3,xdf: p=0.0033).

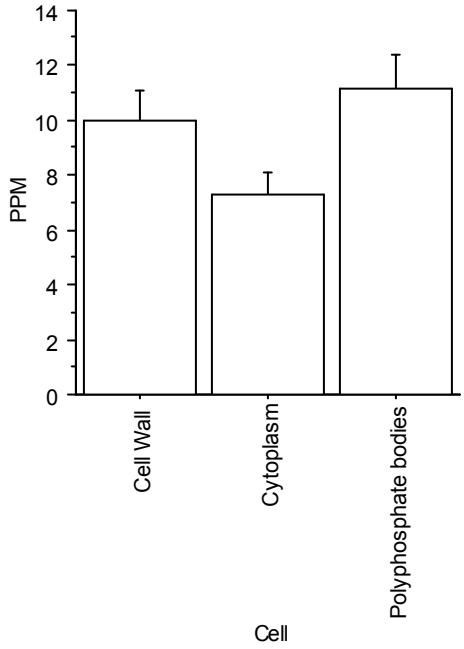



Figure 26: Bar graph for *Synechoccoccus leopoliensis* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA (F=3.689; 3,xdf: p=0.0312).

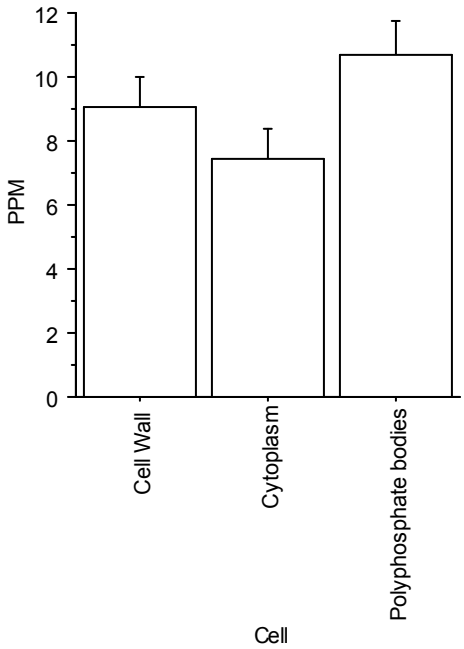



Figure 27: Bar graph for *Synechoccoccus leopoliensis* exposed to Ni. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA (F=2.760; 3,xdf: p=0.0718).

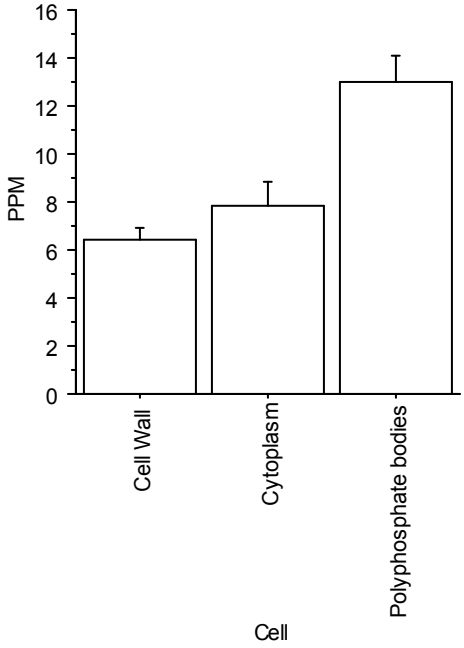



Figure 28: Bar graph for *Synechoccoccus leopoliensis* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA ($F=15.324$; 3,xdf: $p<0.0001$).

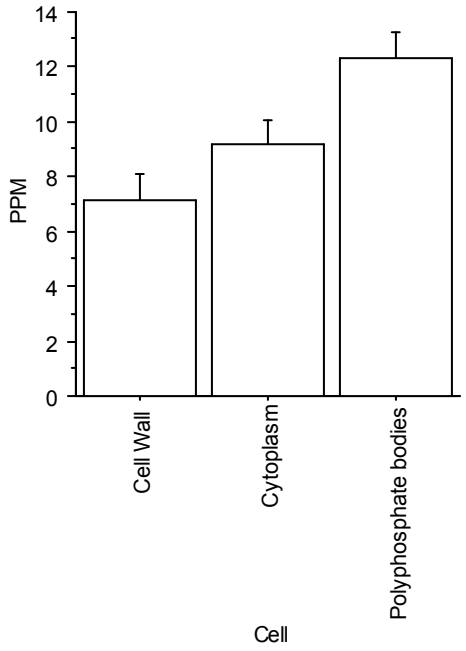



Figure 29: Bar graph for *Synechoccoccus leopoliensis* exposed to Zn. The numbers for the cell means are given in PPMs. ±1SE the means are significant in ANOVA (F=7.967; 3,xdf: p=0.0009).

*Gloeocapsa alpicola*

The cells of *Gloeocapsa alpicola* that were exposed to seven heavy metals independently, the cells showed ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPBs) were analyzed using x-ray analysis. For each heavy metal that the cells were exposed to twenty random cells were analyzed. In addition a control group of *Gloeocapsa alpicola* cells were also analyzed. Figure 30 is an example of a typical x-ray or EDAX spectrum of the polyphosphate body of a *Gloeocapsa alpicola* cell exposed to 5 ppm of Cd. It shows the presence of C, O, K, a range of major elements that are characteristic of this species. Cd is also evident by the peak in the spectrum. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. In each case the control showed no trace of the test metal.

I report here only the amounts of the particular heavy metal that the cells were exposed to. These results were obtained by performing quantitative

analysis on each individual spectra, the mean results are shown in Table 13; parts per million or ppm are equivalent to the values under the column header “Norm Wt.%”(Fig.13-14).

The results from the quantitative analysis of the different cellular components i.e. capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of the Statview 4 and JMP 4. The results from Statview are represented here by the bar plots in Figures 31-37 and the results of the one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 5. This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

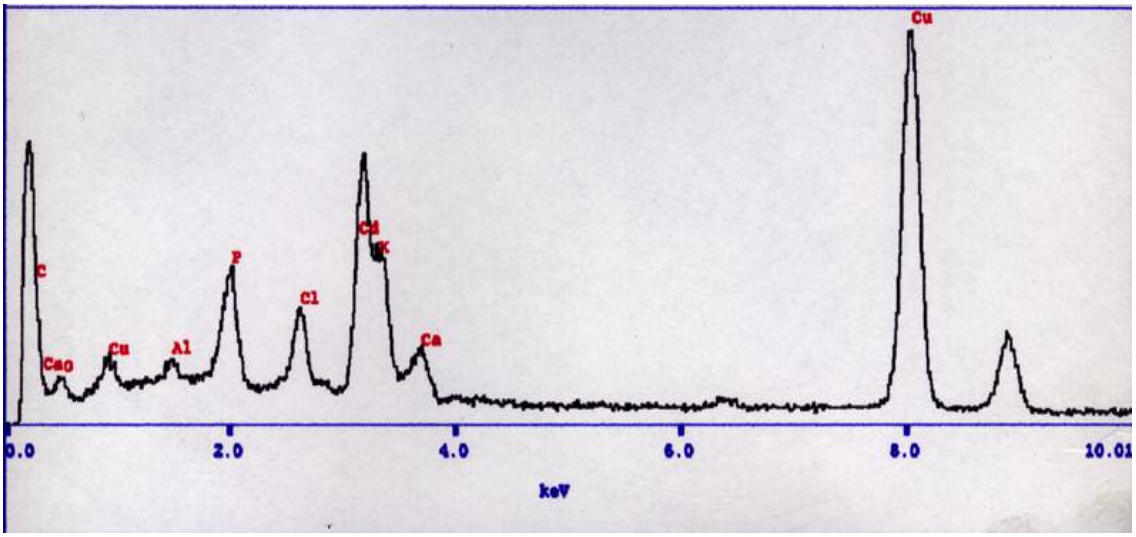



Figure 30: EDAX spectrum of *Gloeocapsa alpicola* PPB exposed to Cd.

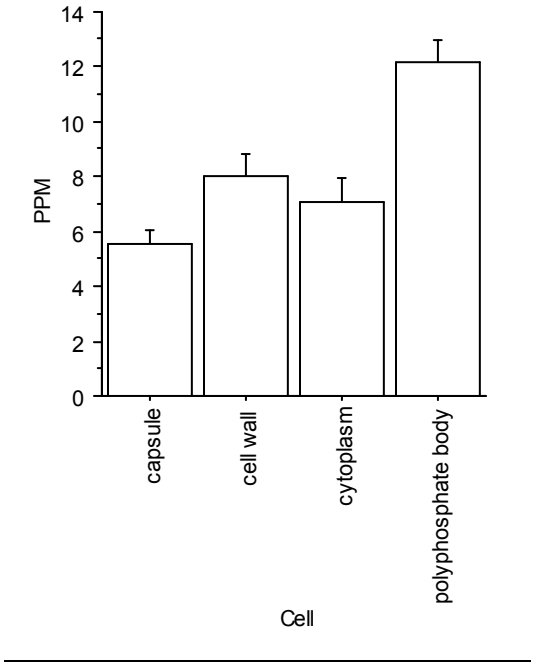



Figure 31: Bar graph for *Gloeocapsa alpicola* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=13.981$; 3,xdf; $p<0.0001$)

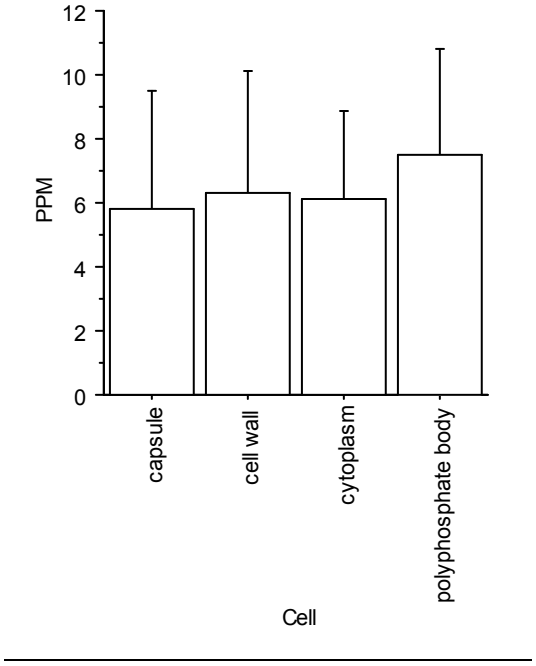



Figure 32: Bar graph for *Gloeocapsa alpicola* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=0.935; 3,xdf; p=0.4283)

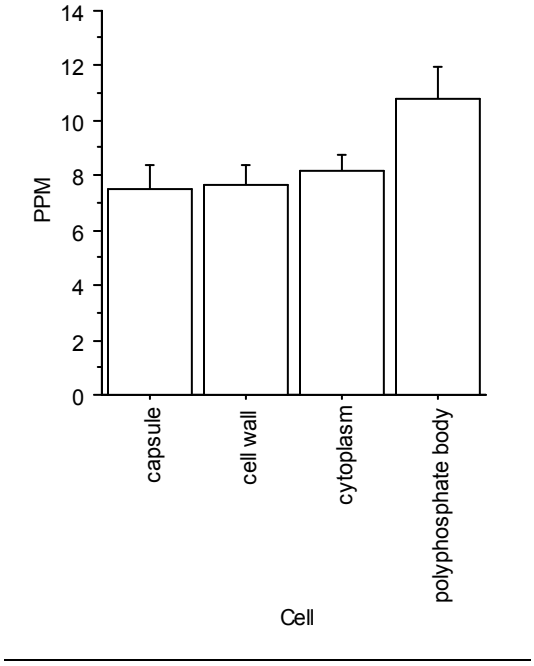



Figure 33: Bar graph for *Gloeocapsa alpicola* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=3.035; 3,xdf; p=0.0335)

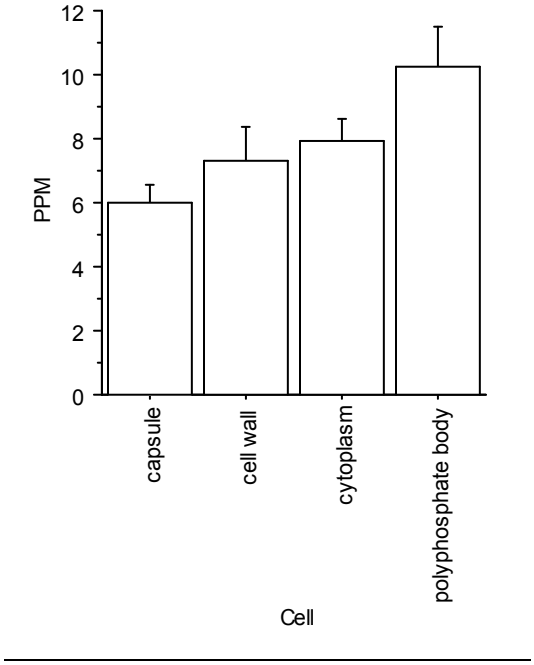



Figure 34: Bar graph for *Gloeocapsa alpicola* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=3.535; 3,xdf; p=0.0187)

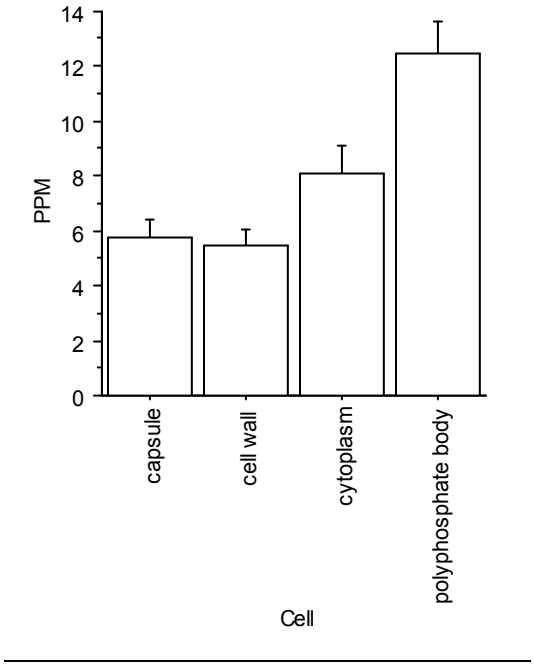



Figure 35: Bar graph for *Gloeocapsa alpicola* exposed to Ni. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=13.644$; 3,xdf; $p<0.0001$)

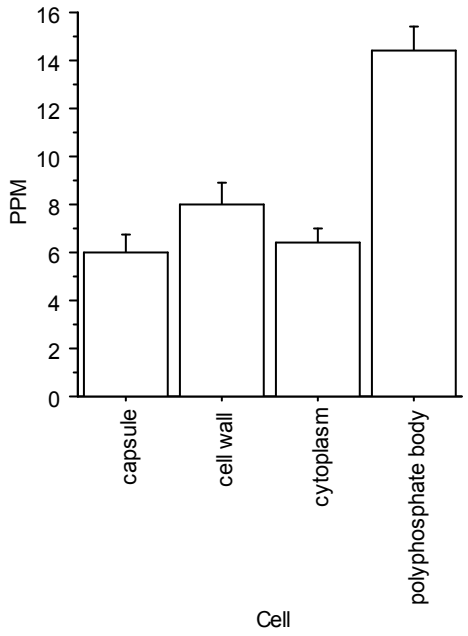



Figure 36: Bar graph for *Gloeocapsa alpicola* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=23.427$; 3,xdf; $p<0.0001$)

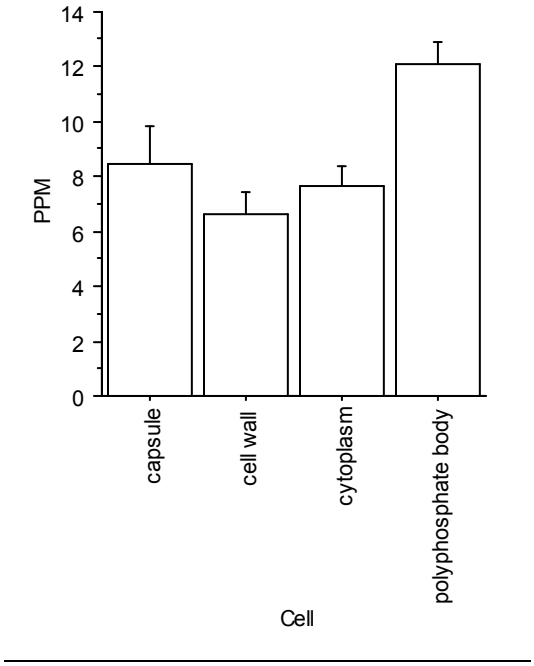



Figure 37: Bar graph for *Gloeocapsa alpicola* exposed to Zn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=6.023; 3,xdf; p=0.0010)

Gram Positive Bacteria

*Bacillus subtilis*

The cells of *Bacillus subtilis* that were exposed to seven heavy metals independently. The cells showed ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPB's) were analyzed using x-ray analysis. For each heavy metal for which the cells were exposed, twenty random cells were analyzed. In addition a control group of *Bacillus subtilis* cells was also analyzed. Figure 38 is an example of a typical x-ray or EDAX spectrum of the cell wall of a *Bacillus subtilis* control cell. It shows the presence of C, O, S, K, Mg, a range of major elements that are characteristic of this species. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. Table 3 shows the mean results of the quantitative analysis done on the EDAX spectrum of twenty test samples of the *Bacillus subtilis* cells. In each case the control showed no trace of the test metal.

I report here only the amounts of the particular heavy metal that the cells were exposed to. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 6; parts per million or ppm are equivalent to the values under the column header “Norm Wt.%”(fig.13-14).

The results from the quantitative analysis of the different cellular components i.e. capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of the Statview 4 and JMP 4. The results are shown in Figures 39-45 and the results of the one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 6.This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

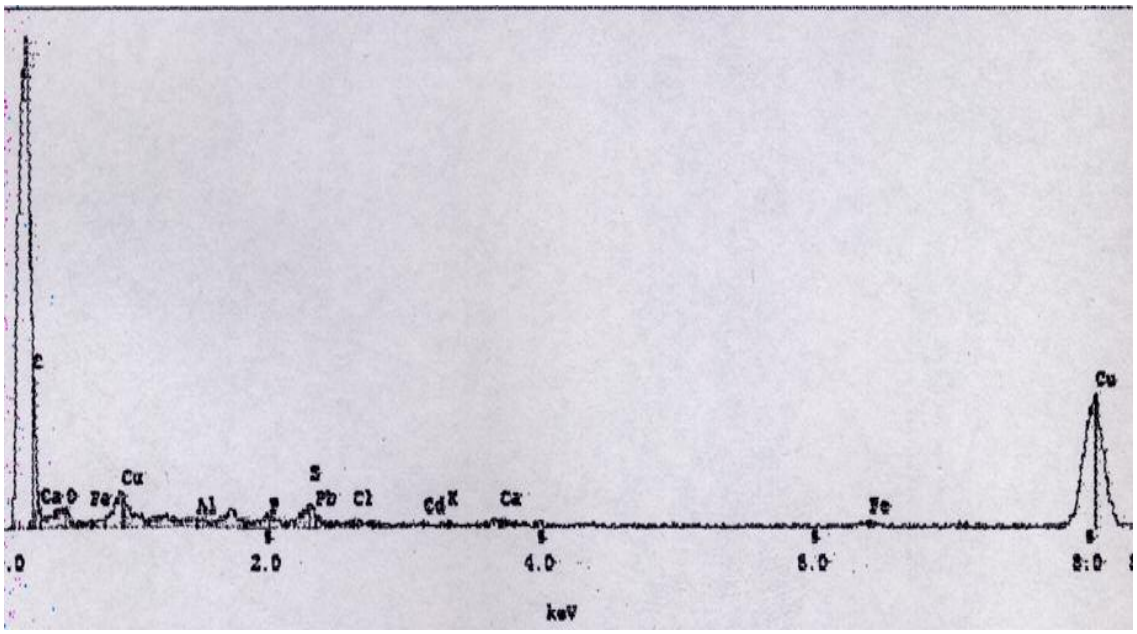



Figure 38: EDAX spectrum of control cytoplasm of *Bacillus subtilis*.

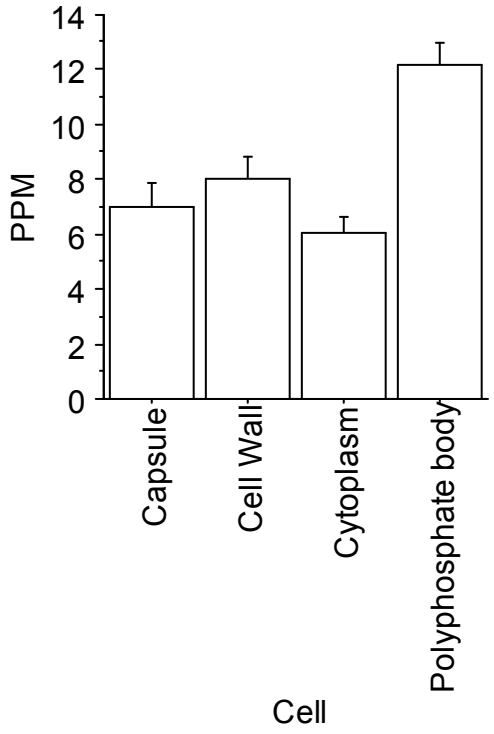



Figure 39: Bar graph for *Bacillus subtilis* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=12.758$; 3,xdf; $p<0.0001$)

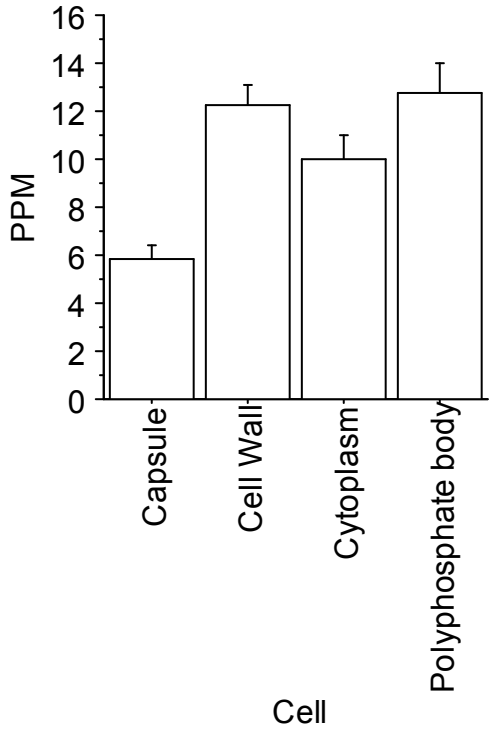



Figure 40: Bar graph for *Bacillus subtilis* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=11.582$; 3,xdf; $p<0.0001$)

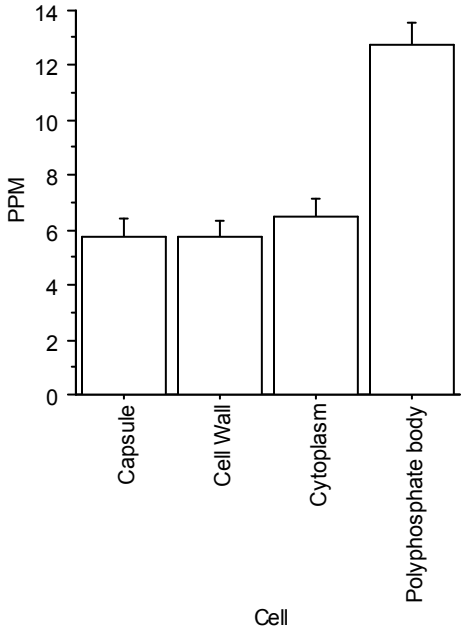



Figure 41: Bar graph for *Bacillus subtilis* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=26.199$; 3,xdf; $p<0.0001$)

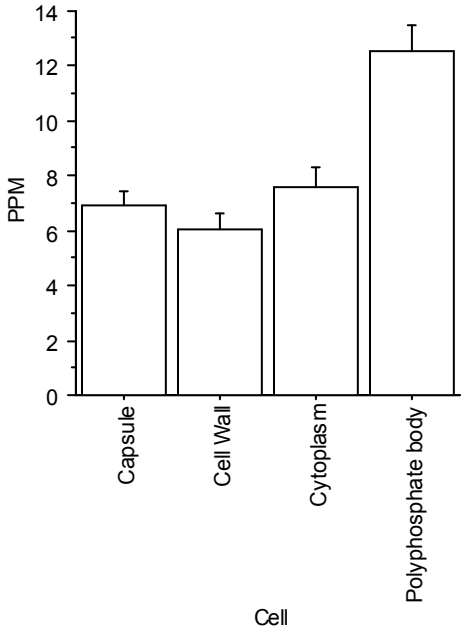



Figure 42: Bar graph for *Bacillus subtilis* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=14.583$; 3,xdf; $p<0.0001$)

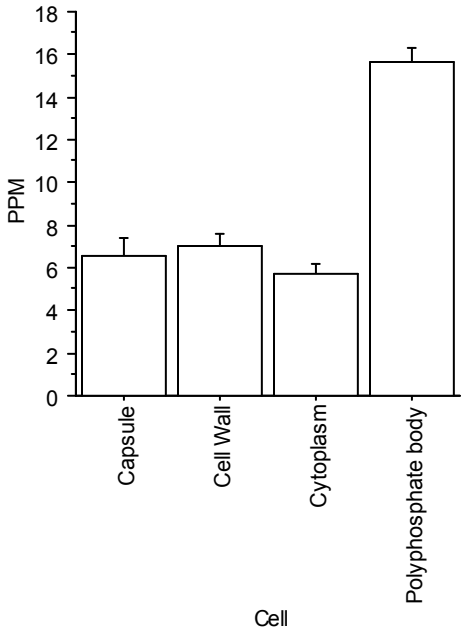



Figure 43: Bar graph for *Bacillus subtilis* exposed to Ni. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=54.417$; 3,xdf; $p<0.0001$)

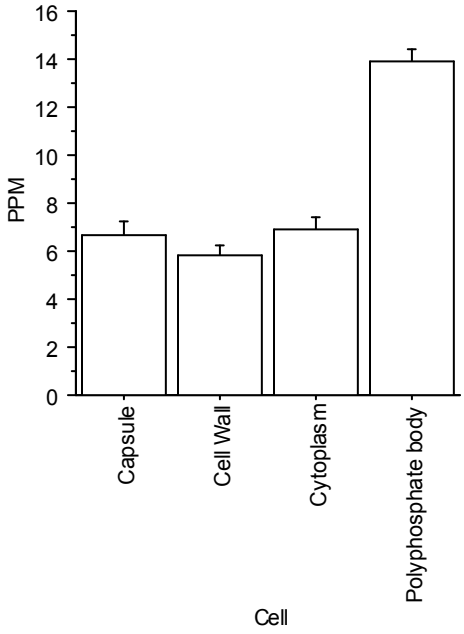



Figure 44: Bar graph for *Bacillus subtilis* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=54.417$; 3,xdf; $p<0.0001$)

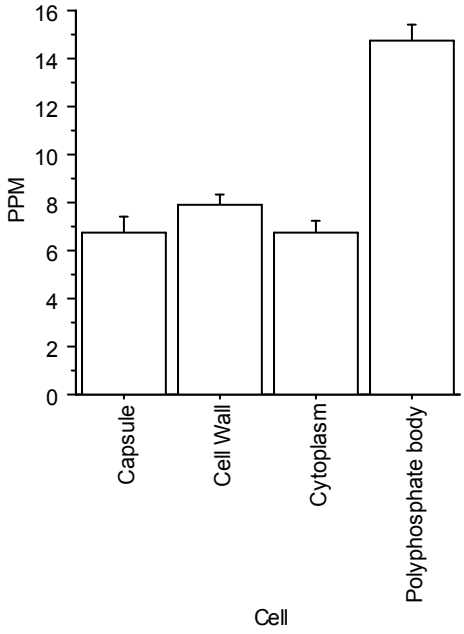



Figure 45: Bar graph for *Bacillus subtilis* exposed to Zn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=0.146$; 3,xdf; $p<0.0001$)

*Staphylococcus aureus*

The cells of *Staphylococcus aureus* that were exposed to seven heavy metals independently. The cells showed ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPB's) were analyzed using x-ray analysis. For each heavy metal that the cells were exposed to twenty random cells were analyzed. In addition a control group of *Staphylococcus aureus* cells were also analyzed. Figure 46 is an example of a typical x-ray or EDAX spectrum of a polyphosphate body of a *Staphylococcus aureus* cell exposed to 20ppm of Zn. It shows the presence of C, O, S, K, a range of major elements that are characteristic of this genus. Zn is also evident by the large peak in the spectrum. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. In each case the control showed no trace of the test metal.

I report here only the amounts of the particular heavy metal that the cells were exposed to. These results were obtained by performing quantitative

analysis on each individual spectra, the mean results are shown in Table 13; parts per million or ppm are equivalent to the values under the column header “Norm Wt.%”(Fig.13-14).

The results from the quantitative analysis of the different cellular components ie.capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of Statview 4 and JMP 4. The results are shown 47-53 and the results of the one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 7.This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

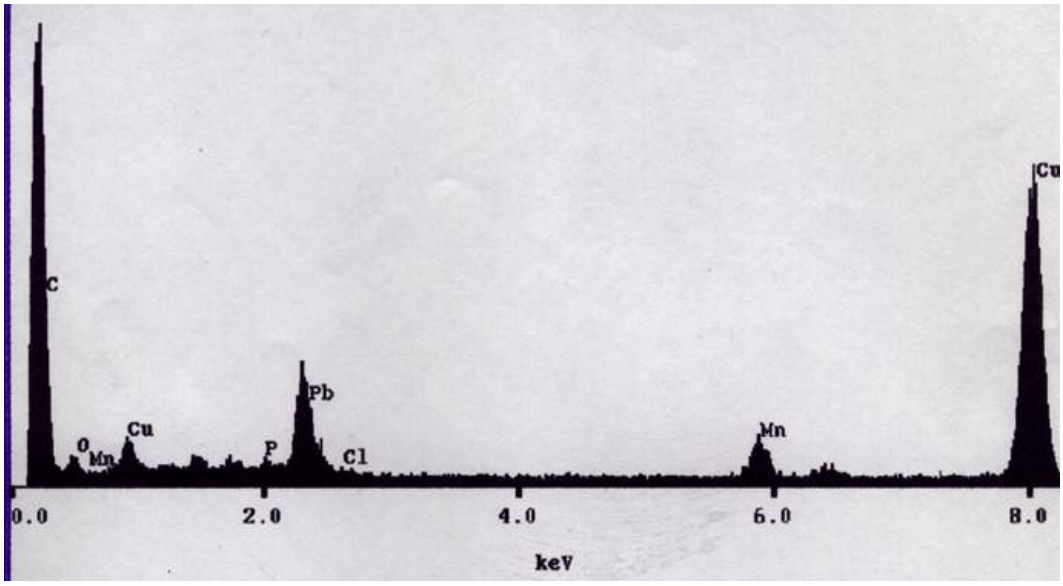



Figure 46: EDAX spectrum of capsule of *Staphylococcus aureus* exposed to Pb.

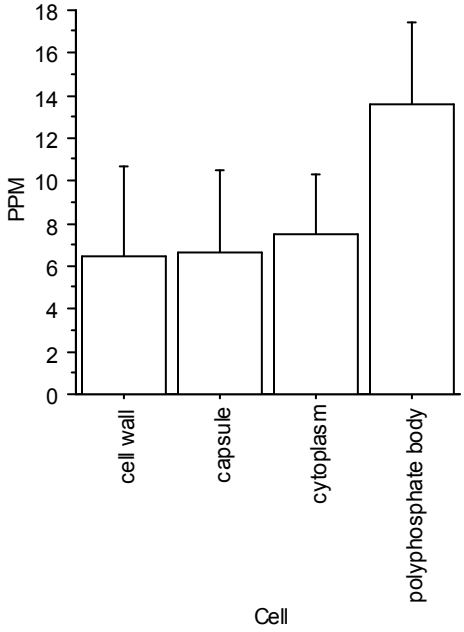



Figure 47: Bar graph for *Staphylococcus aureus* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=16.848$; 3,xdf; $p<0.0001$)

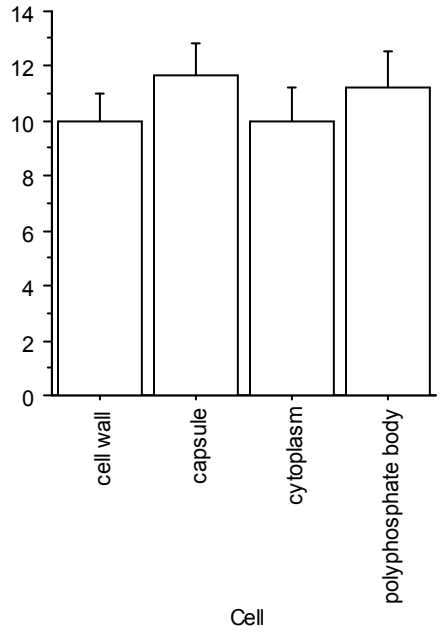



Figure 48: Bar graph for *Staphylococcus aureus* exposed to Cd. The numbers for the cell means are given in PPM's. ±1SE. The means are significant in ANOVA (F=0.498; 3,xdf; p=0.6845).

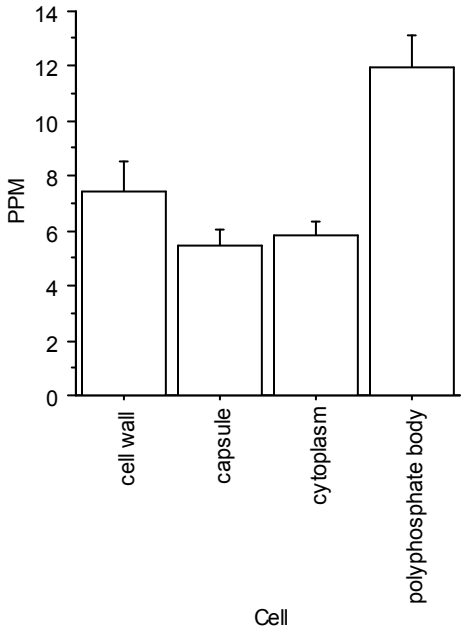



Figure 49: Bar graph for *Staphylococcus aureus* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=11.218; 3,xdf; p<0.0001).

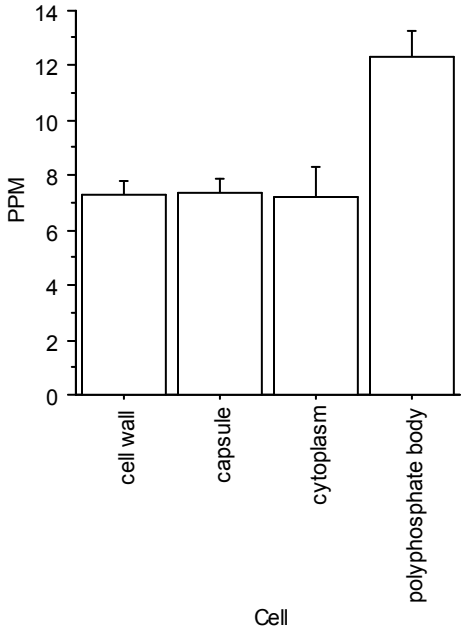



Figure 50: Bar graph for *Staphylococcus aureus* exposed to Mn. The numbers for the cell means are given in PPMs.±1SE. The means are significant in ANOVA (F=9.642; 3,xdf; p<0.0001).

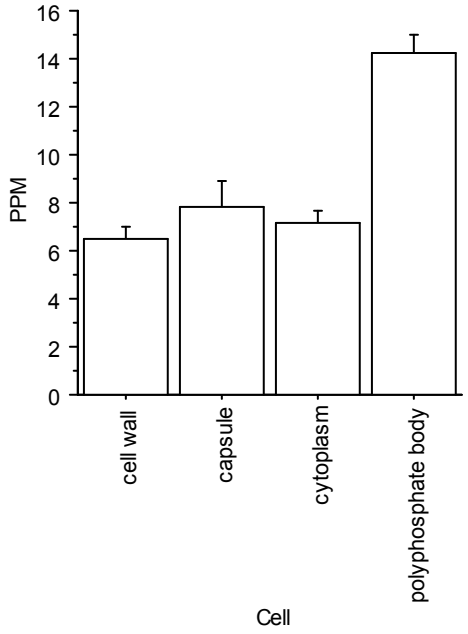



Figure 51: Bar graph for *Staphylococcus aureus* exposed to Ni. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=23.581$; 3,xdf; $p<0.0001$).

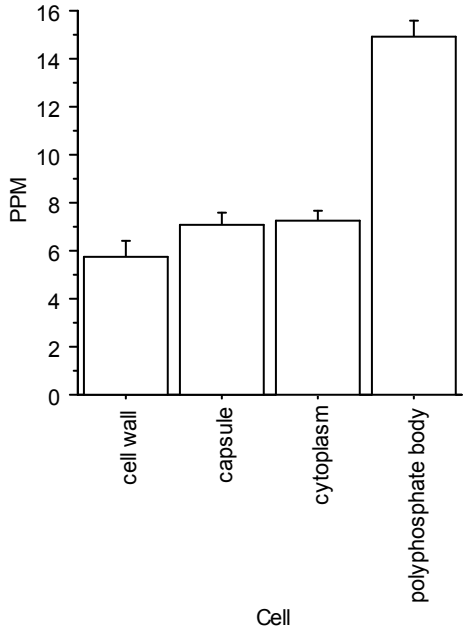



Figure 52: Bar graph for *Staphylococcus aureus* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=55.156$; 3,xdf; $p<0.0001$).

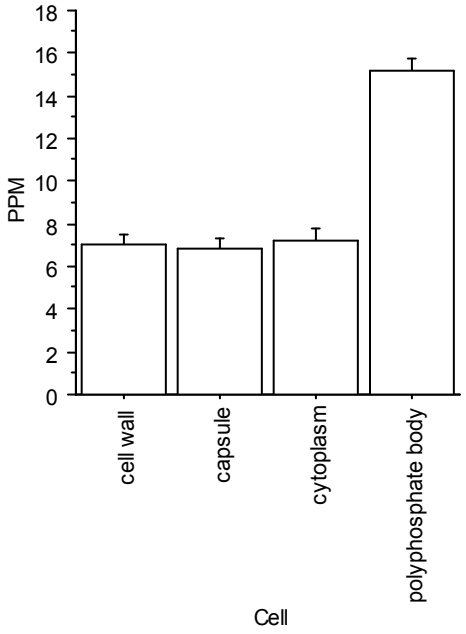



Figure 53: Bar graph for *Staphylococcus aureus* exposed to Zn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=59.366$; 3,xdf; $p<0.0001$).

*Arthrobacter globiformis*

The cells of *Arthrobacter globiformis* were exposed to seven heavy metals independently. The cells showed a ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPB's) were analyzed using x-ray analysis. For each heavy metal that the cells were exposed to twenty random cells were analyzed. In addition a control group of *Arthrobacter globiformis* cells were also analyzed. Figure 54 is an example of a typical x-ray or EDAX spectrum of the polyphosphate body of an *Arthrobacter globiformis* cell exposed to 20 ppm of Pb. It shows the presence of C, O, a range of major elements that and the peak for P are characteristic of this species. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. Figure 13 shows the mean results of the quantitative analysis done on the EDAX spectrum of twenty test samples of the *Arthrobacter globiformis* cells. In each case the control showed no trace of the test metal.

I report here only the amounts of the particular heavy metal that the cells were exposed to. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 13; parts per million or ppm are equivalent to the values under the column header “Norm Wt.%”(Fig.13-14).

The results from the quantitative analysis of the different cellular components ie.capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of Statview 4 and JMP 4. The results 55-61 and the results of the one-way analysis of variance, one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 8.This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

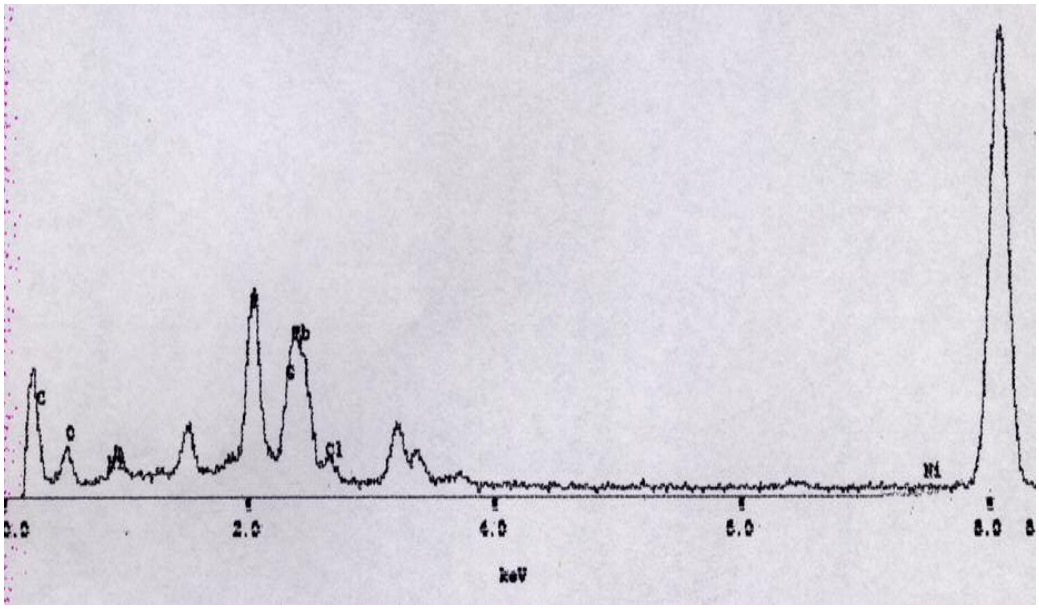



Figure 54: EDAX spectrum of a PPB in a cell of *Arthrobacter globiformis*, exposed to 20 ppm lead.

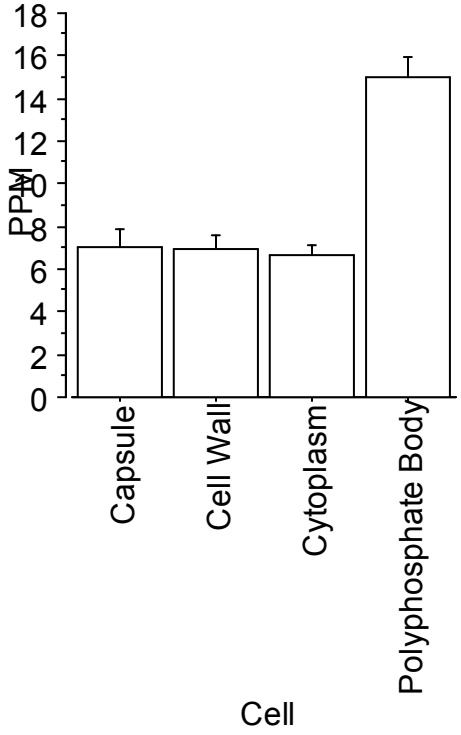



Figure 55: Bar graph for *Arthrobacter globiformis* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=29.746; 3,xdf; p<0.0001).

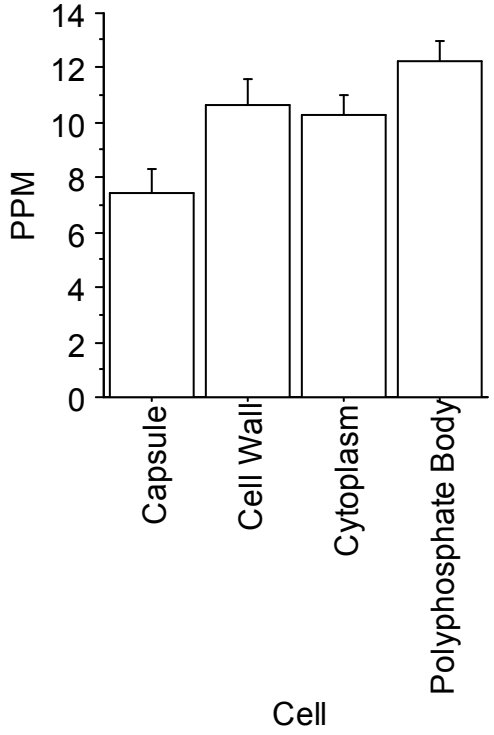



Figure 56: Bar graph for *Arthrobacter globiformis* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=5.764; 3,xdf; p=0.0013).

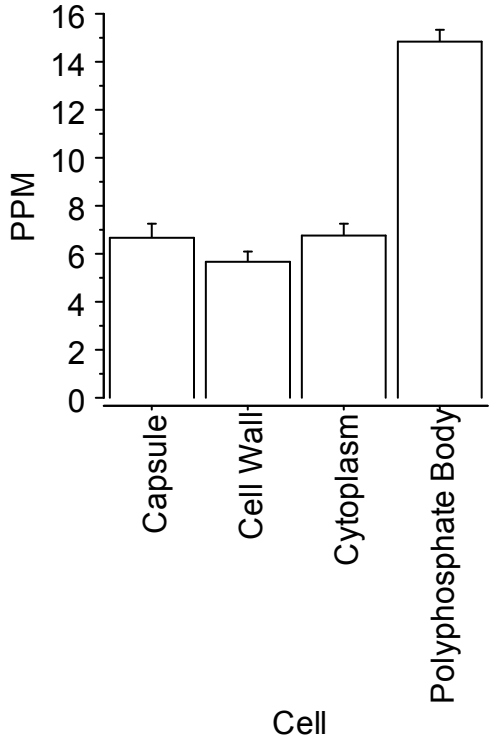



Figure 57: Bar graph for *Arthrobacter globiformis* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=71.315$; 3,xdf; $p<0.0001$).

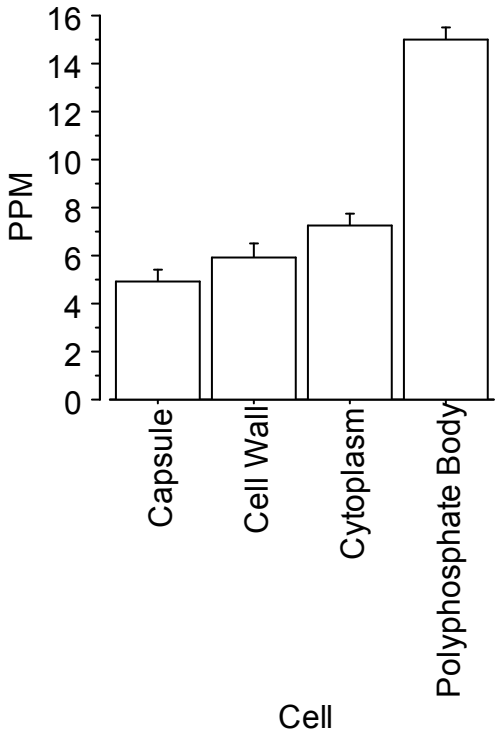



Figure 58: Bar graph for *Arthrobacter globiformis* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=71.995$; 3,xdf; $p<0.0001$).

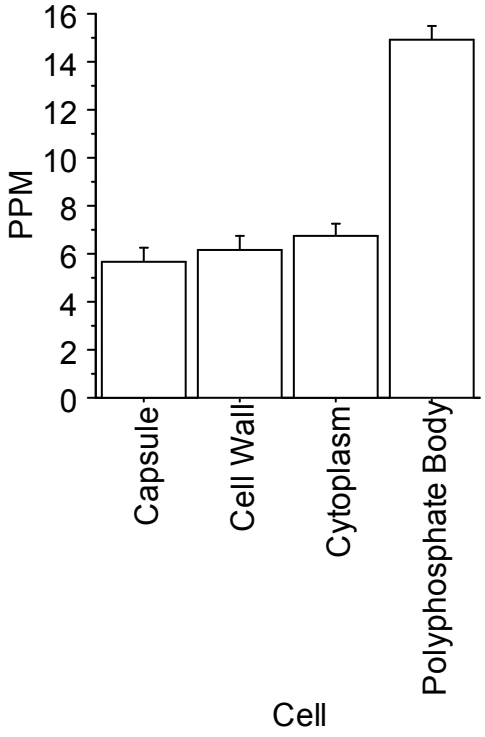



Figure 59: Bar graph for *Arthrobacter globiformis* exposed to Ni. The numbers for thcell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=61.436$; 3,xdf; $p<0.0001$).

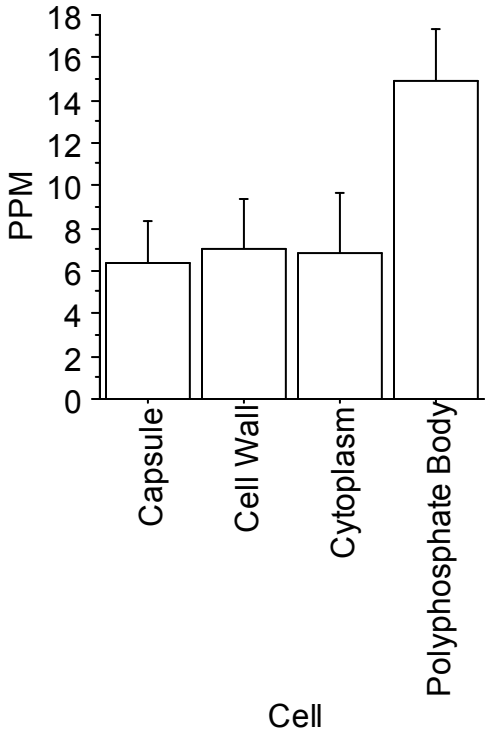



Figure 60: Bar graph for *Arthrobacter globiformis* exposed to Pb The numbers for thcell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=65.930; 3,xdf; p<0.0001).

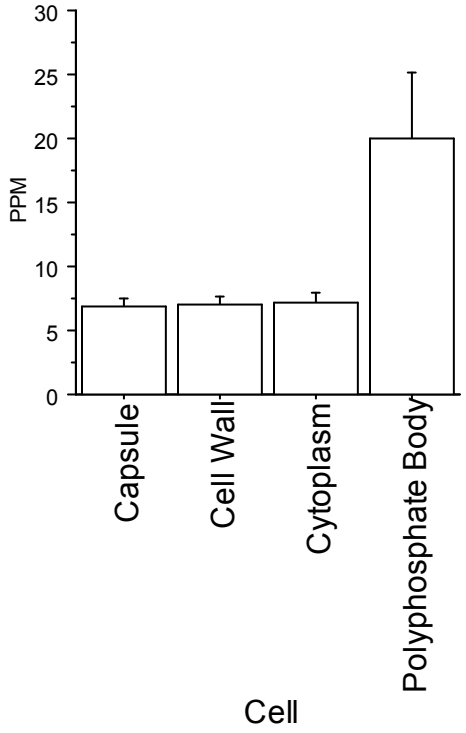



Figure 61: Bar graph for *Arthrobacter globiformis* exposed to Zn The numbers for thcell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=6.272; 3,xdf; p=0.0007).

Gram Negative Bacteria

*Acinetobacter calcoaceticus*

The cells of *Acinetobacter calcoaceticus* were exposed to seven heavy metals independently. The cells showed an ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPB's) were analyzed using x-ray analysis. For each heavy metal that the cells were exposed to twenty random cells were analyzed. In addition a control group of *Acinetobacter calcoaceticus* cells were also analyzed. Figure 62 is an example of a typical x-ray or EDAX spectrum of the PPB inclusion of *Acinetobacter calcoaceticus* cell exposed to 20 ppm of Al. It shows the presence of C, O, S, K, Mg, a range of major elements that are characteristic of this species. Al is also evident by the peak in the spectrum. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism.

Figure 3 shows the mean results of the quantitative analysis done on the EDAX spectrum of twenty test samples of the *Acinetobacter calcoaceticus* cells. In each case the control showed no trace of the test metal.

I report here only the amounts of the particular heavy metal for which the cells were exposed to. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 13; parts per million or ppm are equivalent to the values under the column header “Norm Wt.%”(Fig.13-14).

The results from the quantitative analysis of the different cellular components ie.capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of the Statview 4 and JMP 4. The results 63-69 and the results of the one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 9. This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

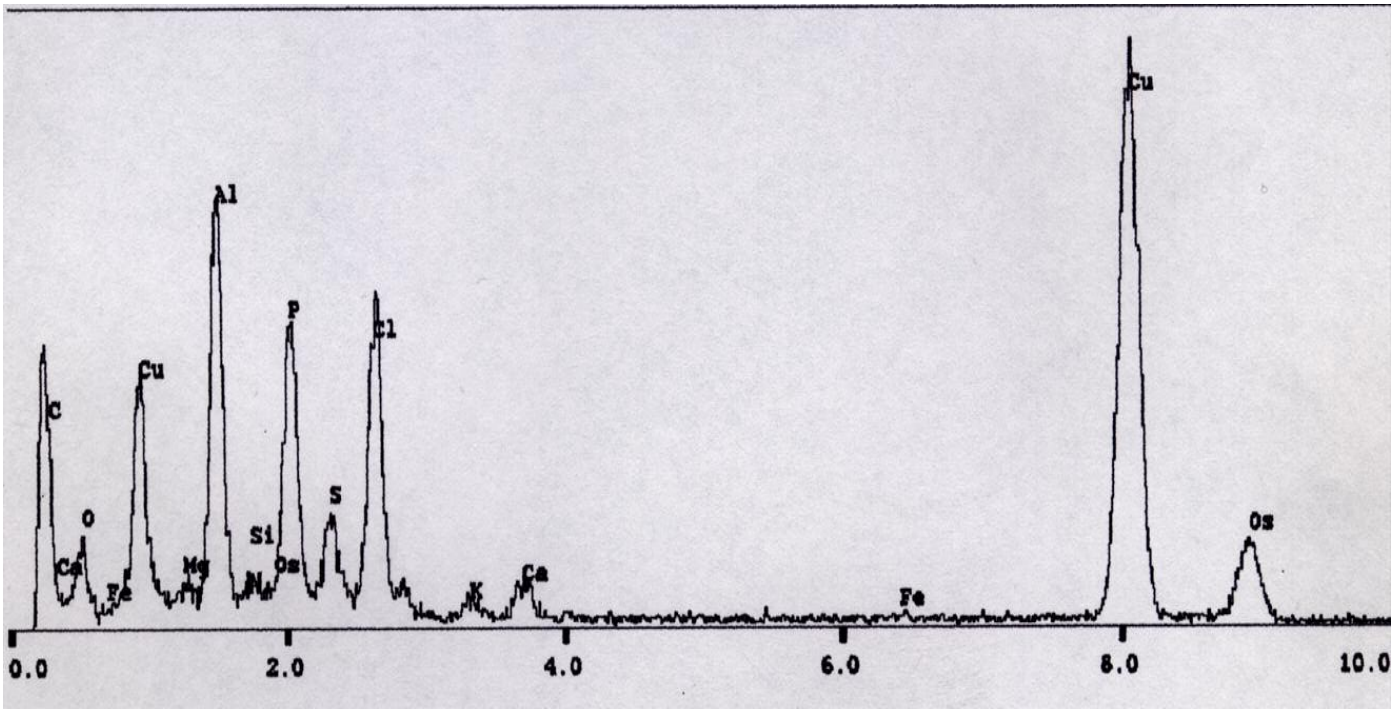



Figure 62: EDAX spectrum of PPB of a sectioned cell of *Acinetobacter calcoaceticus*, exposed to 20 ppm of Al.

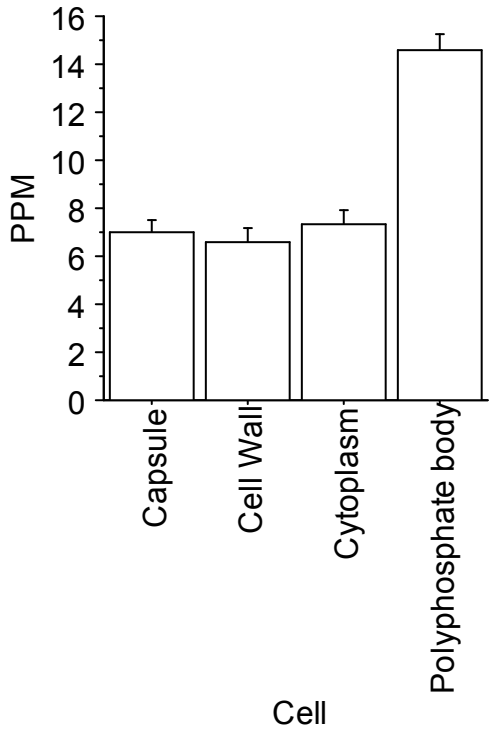



Figure 63: Bar graph for *Acinetobacter calcoaceticus* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=41.850$; 3,xdf; $p<0.0001$).

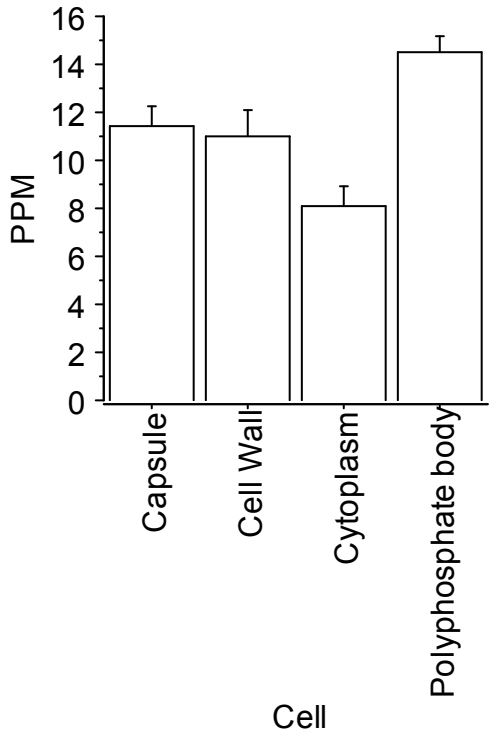



Figure 64: Bar graph for *Acinetobacter calcoaceticus* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=9.920$; 3,xdf; $p<0.0001$).

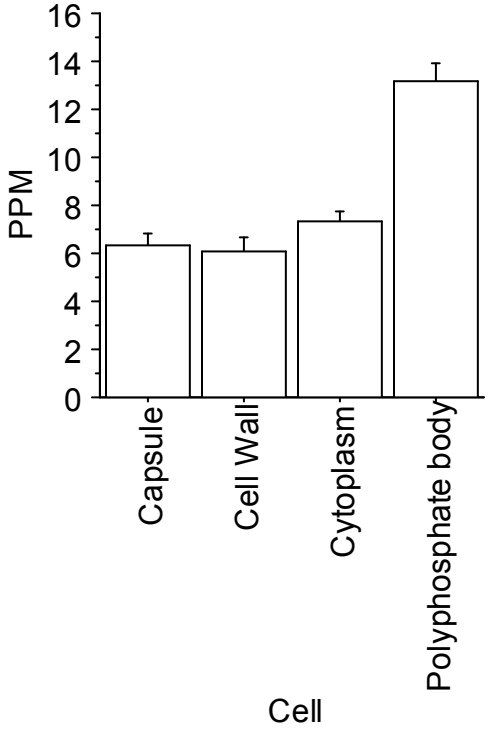



Figure 65: Bar graph for *Acinetobacter calcoaceticus* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=34.537$; 3,xdf; $p<0.0001$).

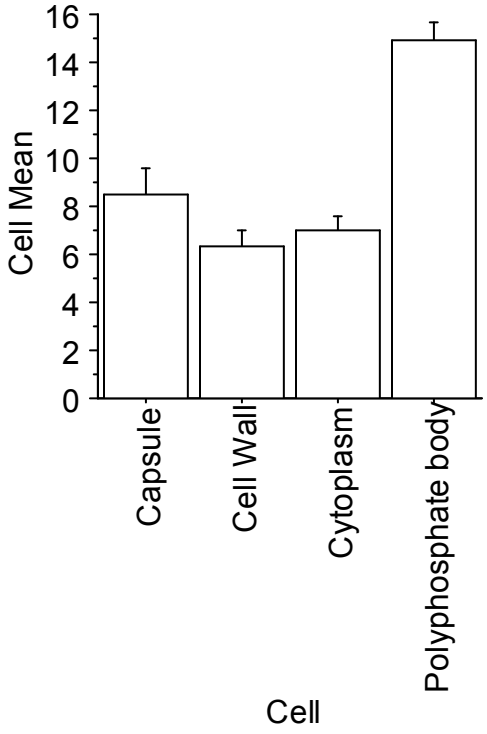



Figure 66: Bar graph for *Acinetobacter calcoaceticus* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=24.454$; 3,xdf; $p<0.0001$).

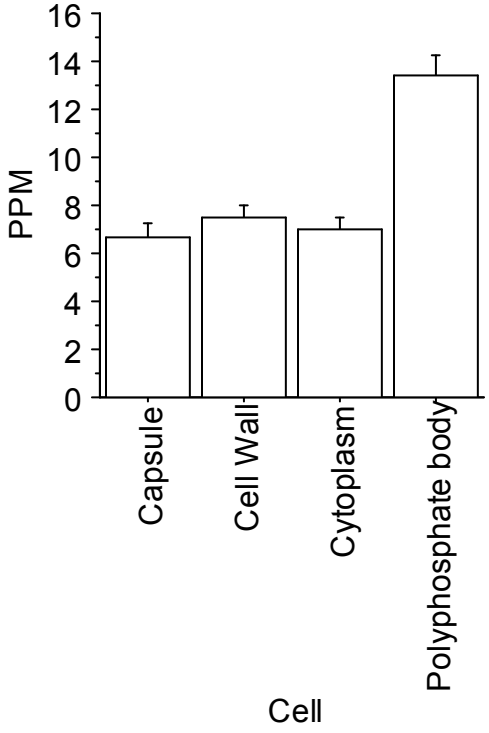



Figure 67: Bar graph for *Acinetobacter calcoaceticus* exposed to Ni. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=25.848$; 3,xdf; $p<0.0001$).

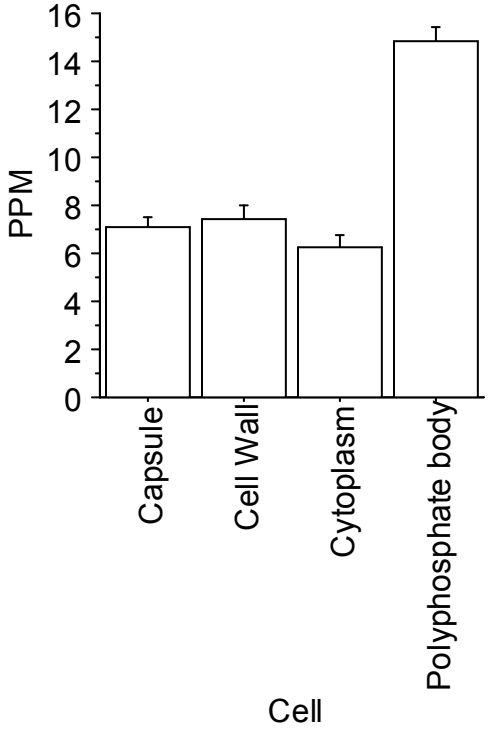



Figure 68: Bar graph for *Acinetobacter calcoaceticus* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=48.190$; 3,xdf; $p<0.0001$).

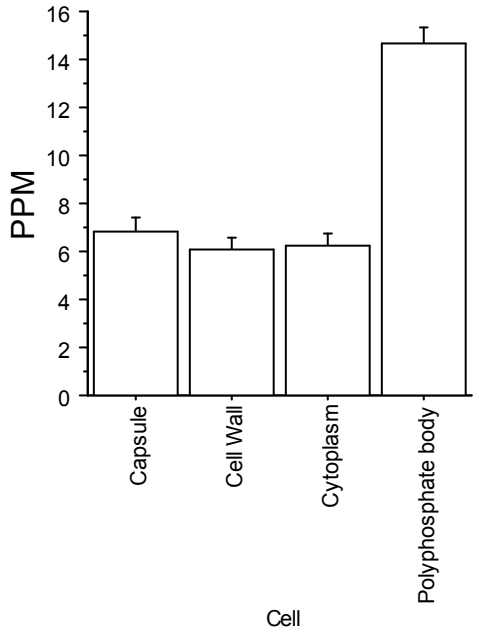



Figure 69: Bar graph for *Acinetobacter calcoaceticus* exposed to Zn. The numbers for the cell means are given in PPM's. ±1SE. The means are significant in ANOVA ($F=52.334$; 3,xdf; $p<0.0001$).

*Pseudomonas aeruginosa*

The cells of *Pseudomonas aeruginosa that* were exposed to seven heavy metals independently, the cells showed an ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPB's) were analyzed using x-ray analysis. For each heavy metal that the cells were exposed to twenty random cells were analyzed. In addition a control group of *Pseudomonas aeruginosa* cells were also analyzed. Figure 70 is an example of a typical x-ray or EDAX spectrum of the cytoplasm of a *Pseudomonas aeruginosa* cell exposed to Cu. It shows the presence of C, O, S, K, Cl, a range of major elements that are characteristic of this species. Cu is also evident by the large peak in the spectrum. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. In each case the control showed no trace of the test metal.

Figure 13 shows the mean results of the quantitative analysis done I report here only the amounts of the particular heavy metal that the cells were

exposed to. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 13; parts per million or ppm are equivalent to the values under the column header “Norm Wt.%”(Fig.13-14).

The results from the quantitative analysis of the different cellular components i.e. capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of the Statview 4 program and JMP 4. The results 71-77 and the results of the one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 10.This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

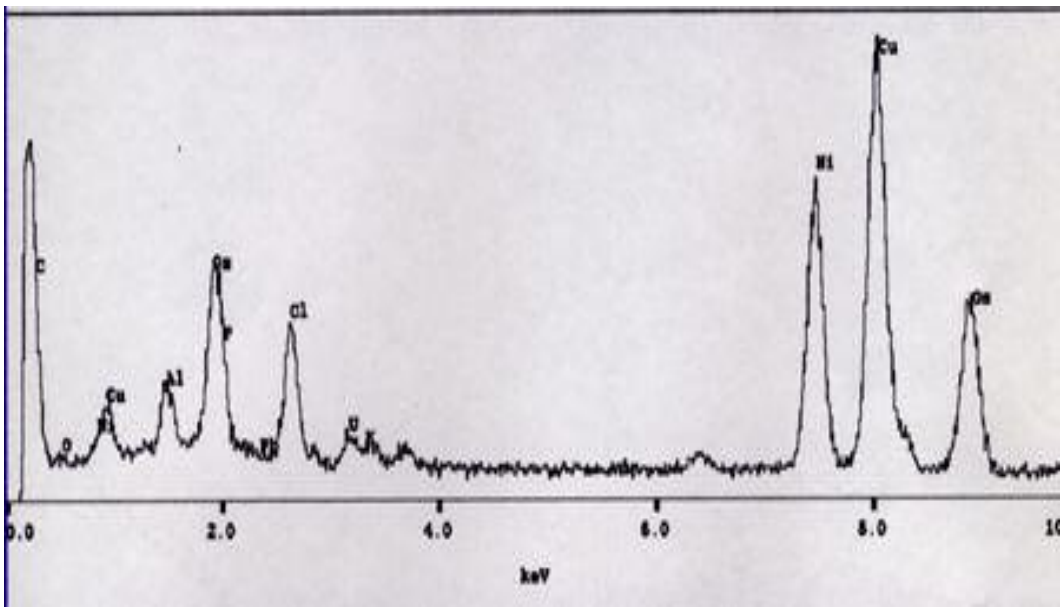



Figure 70: EDAX spectrum of cytoplasm of *Pseudomonas aeruginosa*, exposed to 20 ppm of Cu notice the Ni peak coincides with the use of nickel mesh grids to do the analysis.

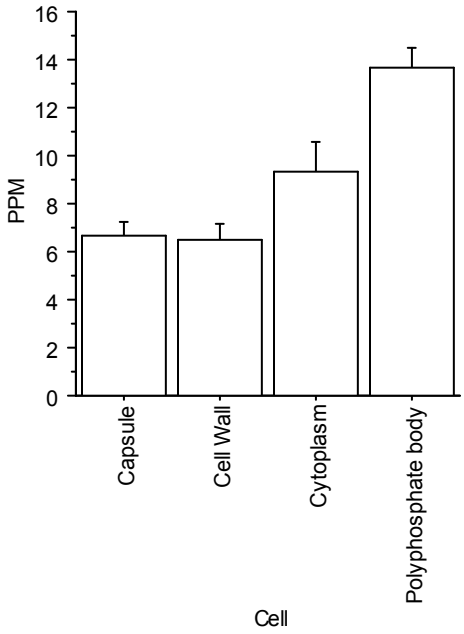



Figure 71: Bar graph for *Pseudomonas aeruginosa* exposed to AL. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=14.265$; 3,xdf; $p<0.0001$).

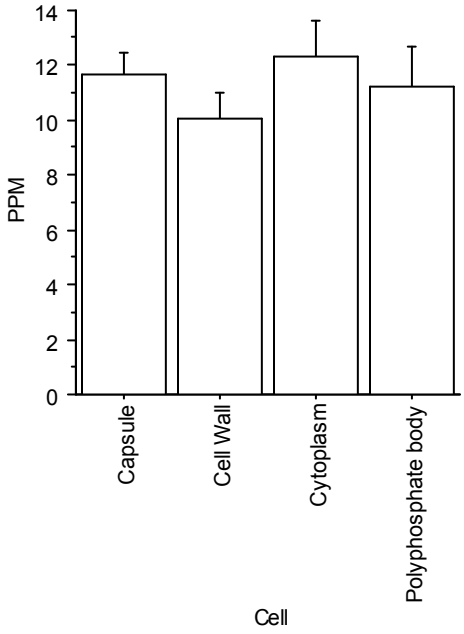



Figure 72: Bar graph for *Pseudomonas aeruginosa* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=17.528; 3,xdf; p=0.5934).

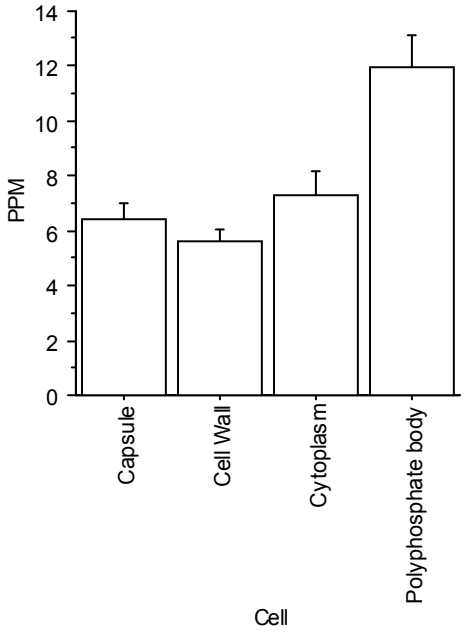



Figure 73: Bar graph for *Pseudomonas aeruginosa* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=13.169; 3,xdf; p<0.0001).

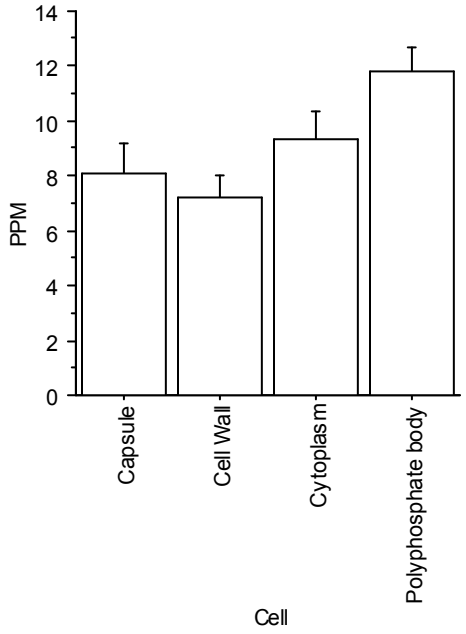



Figure 74: Bar graph for *Pseudomonas aeruginosa* exposed to Mn The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=4.078; 3,xdf; p=0.0097).

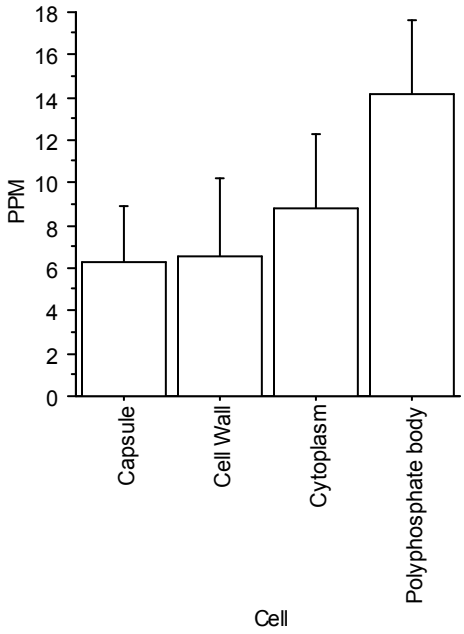



Figure 75: Bar graph for *Pseudomonas aeruginosa* exposed to Ni. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=24.418$; 3,xdf; $p<0.0001$).

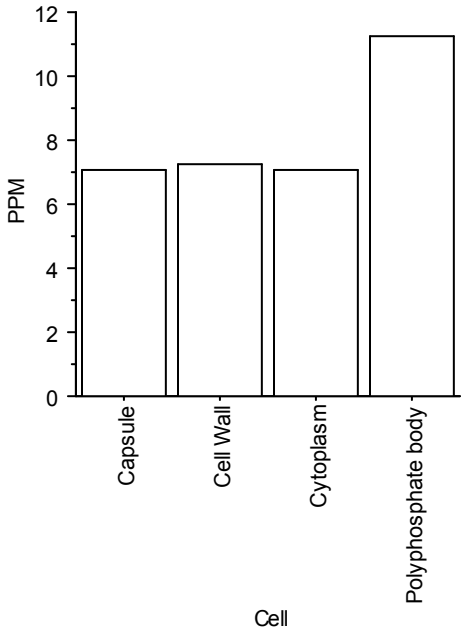



Figure 76: Bar graph for *Pseudomonas aeruginosa* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=5.683; 3,xdf; p=0.0014).

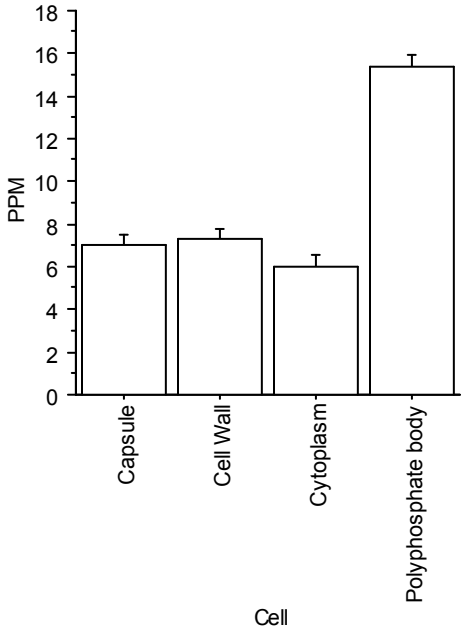



Figure 77: Bar graph for *Pseudomonas aeruginosa* exposed to Zn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=70.265; 3,xdf; p=0.0001).

*Escherichia coli*

The cells of *Escherichia coli* were exposed to seven heavy metals independently, the cells showed ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPBs) were analyzed using x-ray analysis. For each heavy metal that the cells were exposed to twenty random cells were analyzed. In addition a control group of Escherichia coli cells was also analyzed. Figure 78 is an example of a typical x-ray or EDAX spectrum of the PPB inclusion of a control cell of *Escherichia coli*. It shows the presence of C, O, S, K, Si, Al a range of major elements that are characteristic of this species. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. In each case the control showed no trace of the test metal.

Figure 13 shows the mean results of the quantitative analysis done on the EDAX spectrum of twenty test samples of the *Escherichia coli* cells. I report here only the amounts of the particular heavy metal that the cells were exposed to. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 13; parts per

million or ppm are equivalent to the values under the column header “Norm Wt.%”(Fig13-14).

The results from the quantitative analysis of the different cellular components i.e. capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of the Statview 4 and JMP 4. The results 48-54 and the results of the one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 11. This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

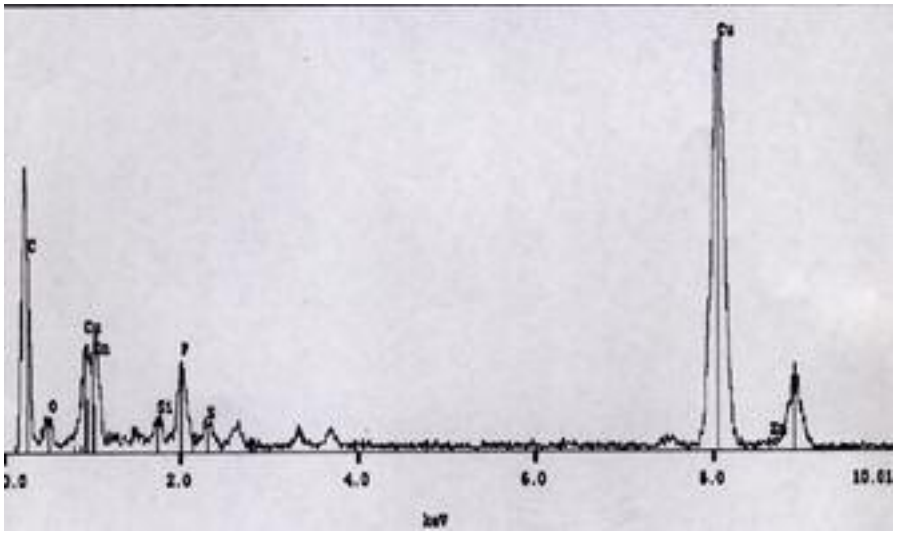



Figure 78: EDAX spectrum of cell capsule of *Escherichia coli.* Exposed to 20 ppm of Zn.

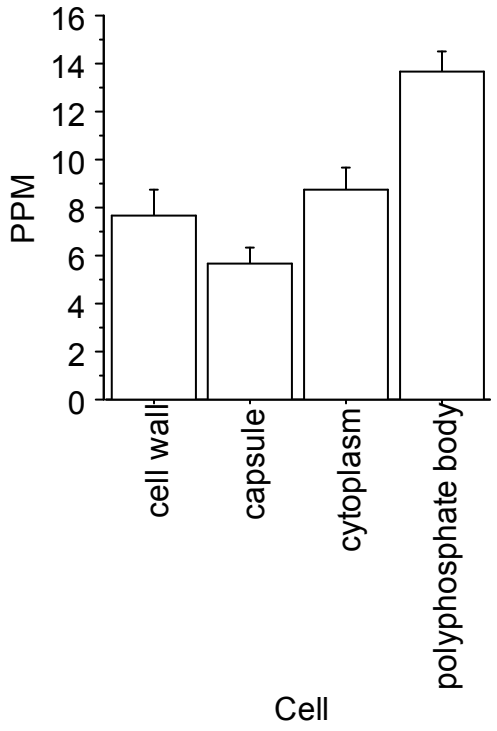



Figure 79: Bar graph for *Escherichia coli* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=14.412$; 3,xdf; $p<0.0001$).

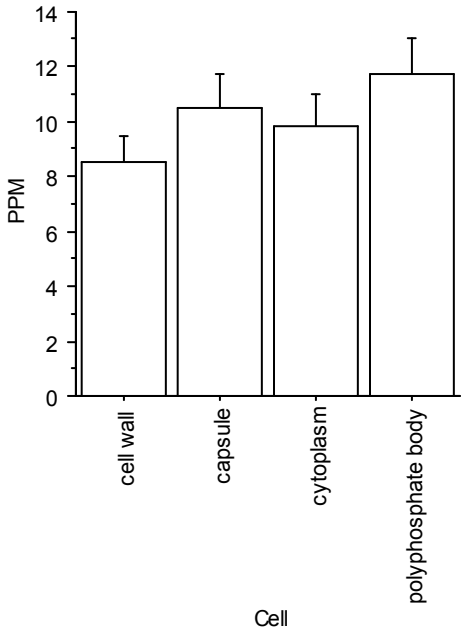



Figure 80: Bar graph for *Escherichia coli* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=1.258; 3,xdf; p=0.2958).

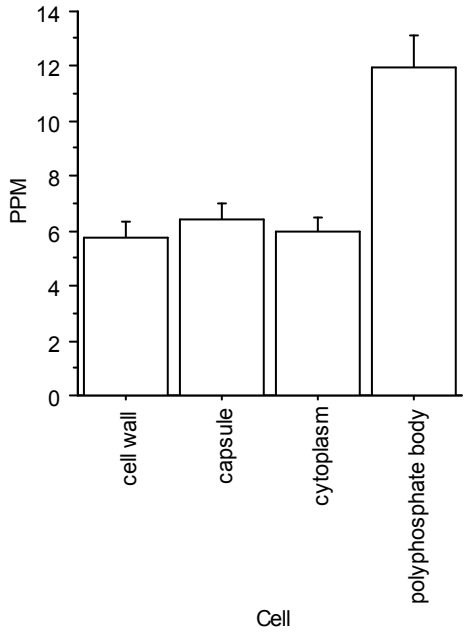



Figure 81: Bar graph for *Escherichia coli* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=15.878$; 3, xdf; $p<0.0001$).

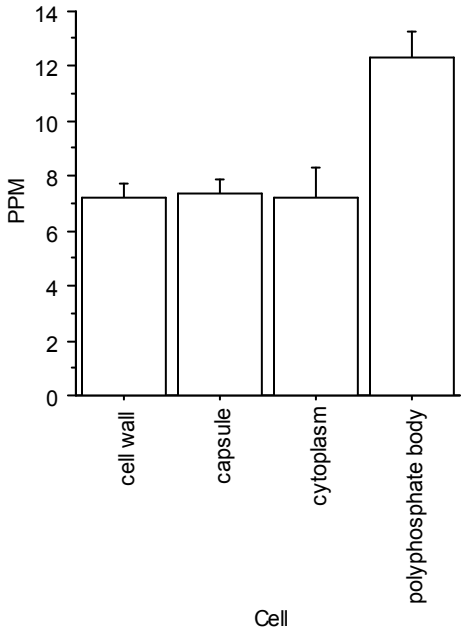



Figure 82: Bar graph for *Escherichia coli* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=9.708$; 3, xdf; $p<0.0001$).

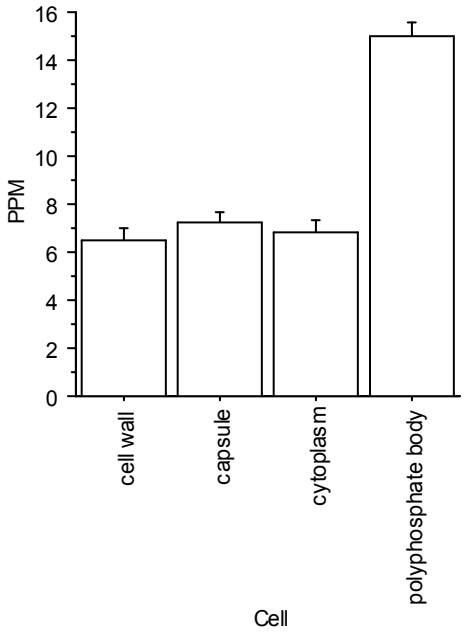



Figure 83: Bar graph for *Escherichia coli* exposed to Ni. The numbers for the cell means are given in PPM's. ±1SE. The means are significant in ANOVA ($F=67.142$; 3, xdf; $p<0.0001$).

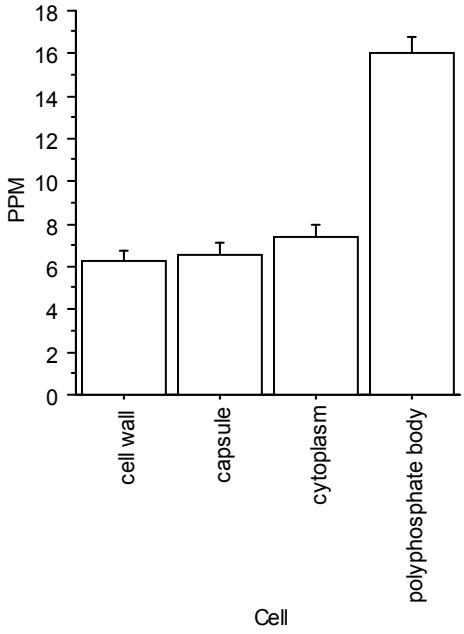



Figure 84: Bar graph for *Escherichia coli* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=66.845$; 3, xdf; $p<0.0001$).

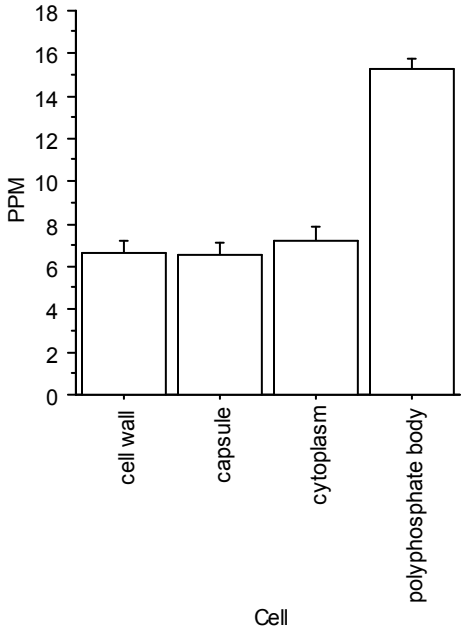



Figure 85: Bar graph for *Escherichia coli* exposed to Zn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=61.042$; 3, xdf; $p<0.0001$).

Eukaryotic Organisms (Yeasts)

*Saccharomyces cerevisiae*

The cells of *Saccharomyces cerevisiae* were exposed to seven heavy metals independently. The cells showed an ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPB's) were analyzed using x-ray analysis. For each heavy metal that the cells were exposed to twenty random cells were analyzed. In addition a control group of *Saccharomyces cerevisiae* cells was also analyzed. Figure 86 is an example of a typical x-ray or EDAX spectrum of the PPB inclusion of a control cell of *Saccharomyces cerevisiae.* It shows the presence of C, O, S, K, Si, Al a range of major elements that are characteristic of this species. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. Figure 13 shows the mean results of the quantitative analysis done on the EDAX spectrum of twenty test samples of the *Saccharomyces cerevisiae* cells. In each case the control showed no trace of the test metal.

I report here only the amounts of the particular heavy metal that the cells were exposed to. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 13; parts per million or ppm are equivalent to the values under the column header “Norm Wt.%”(Fig.13-14).

The results from the quantitative analysis of the different cellular components ie.capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of Statview 4 and JMP 4. The results 87-93 and the results of the one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 12.This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

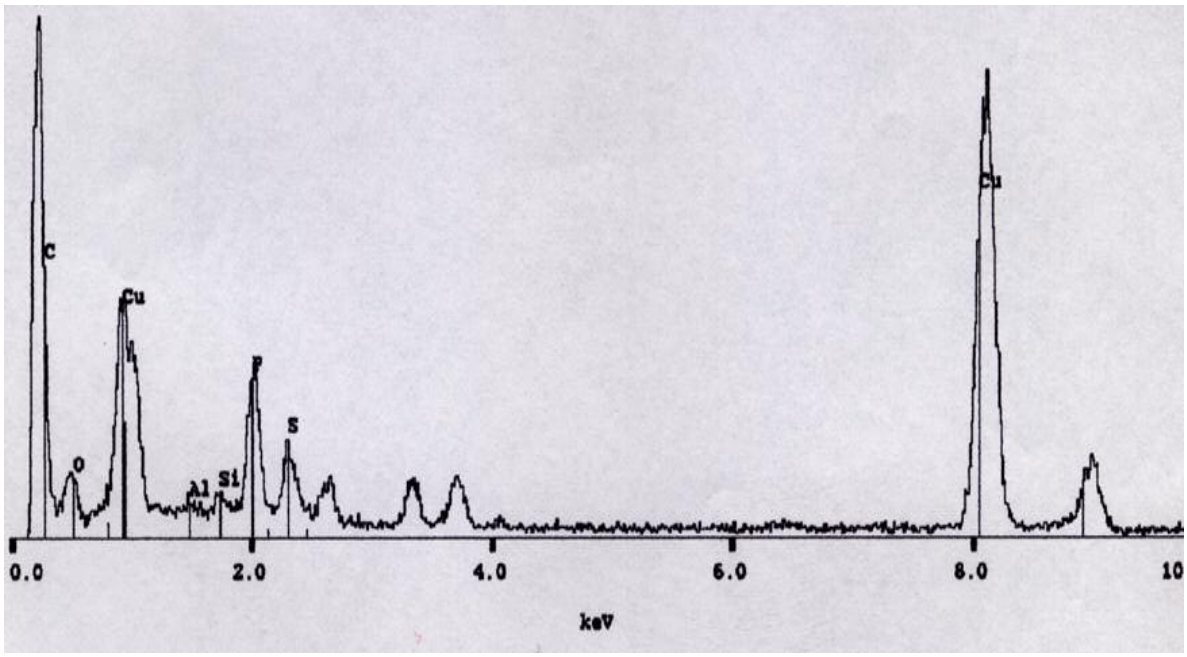



Figure 86: EDAX spectrum of control PPB from a cell of *Sacchromyces cerviciae.*

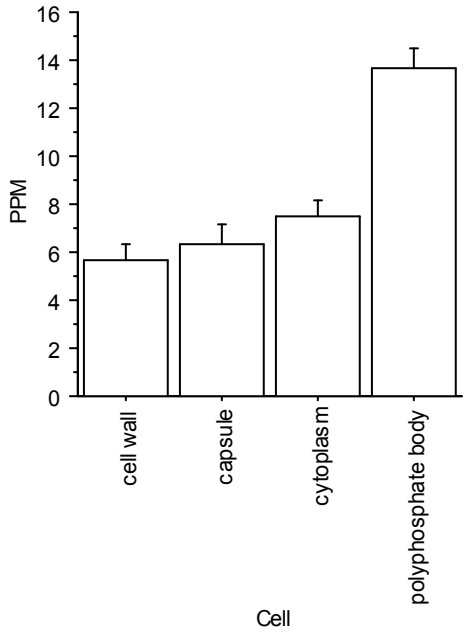



Figure 87: Bar graph for *Sacchromyces cerviciae* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=23.843$; 3, xdf; $p<0.0001$).

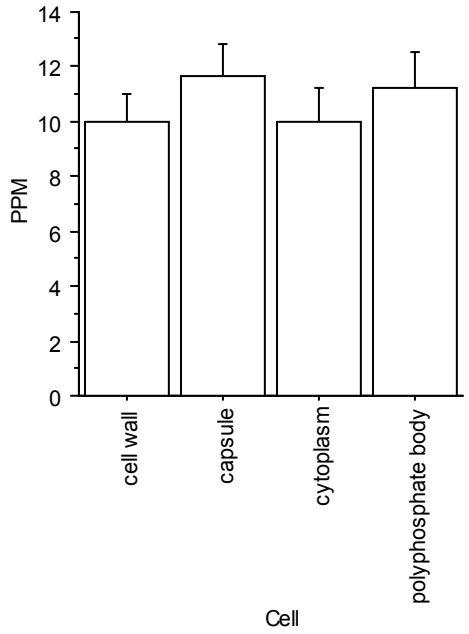



Figure 88: Bar graph for *Sacchromyces cerviciae* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=0.498; 3, xdf; p=0.6845).

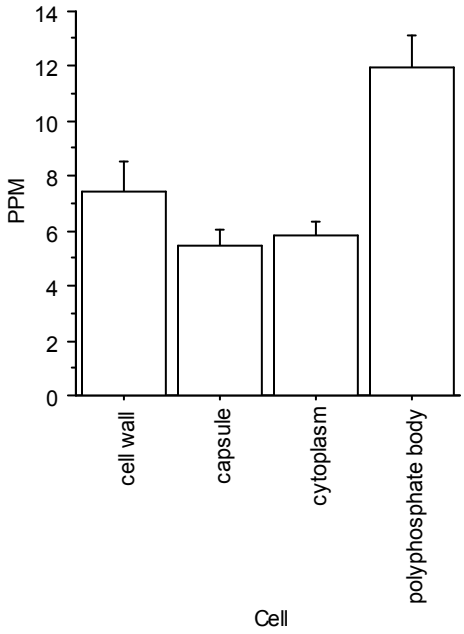



Figure 89: Bar graph for *Sacchromyces cerviciae* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA (F=11.218; 3, xdf; p<0.0001).

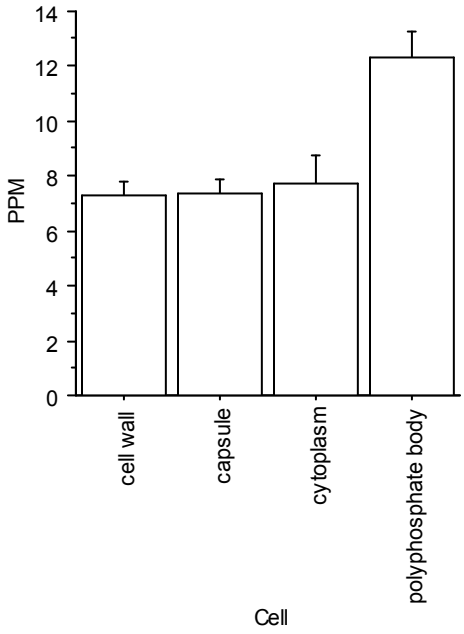



Figure 90: Bar graph for *Sacchromyces cerviciae* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=9.224$; 3, xdf; $p<0.0001$).

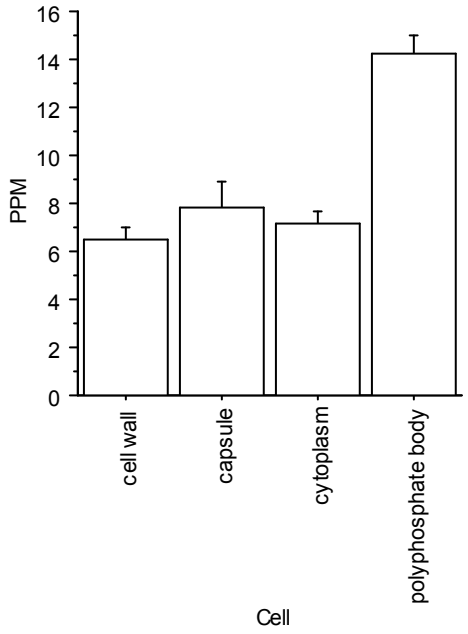



Figure 91: Bar graph for *Sacchromyces cerviciae* exposed to Ni. The numbers for the cell means are given in PPM's. ±1SE. The means are significant in ANOVA (F=23.581; 3, xdf; p<0.0001).

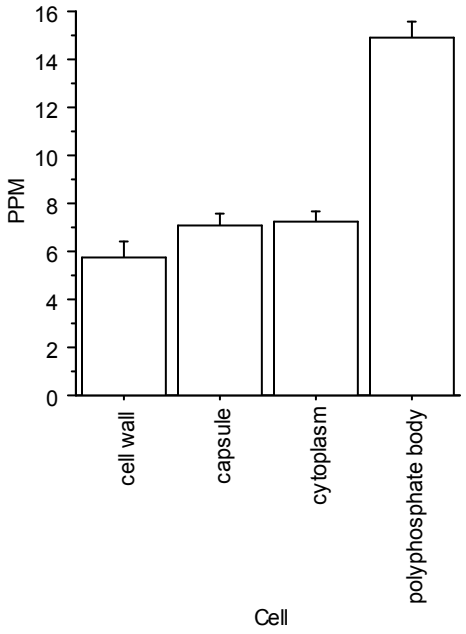



Figure 92: Bar graph for *Sacchromyces cerviciae* exposed to Pb. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=55.156$; 3, xdf; $p<0.0001$).

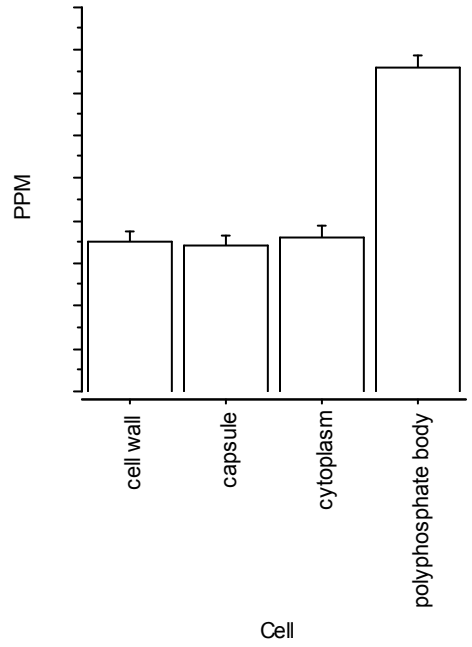



Figure 93: Bar graph for *Sacchromyces cerviciae* exposed to Zn. The numbers for the cell means are given in PPM's. ±1SE. The means are significant in ANOVA ($F=59.366$; 3, xdf; $p<0.0001$).

*Rhodotorula rubra*

The cells of *Rhodotorula rubra* were exposed to seven heavy metals independently, the cells showed ability to uptake metal in different areas or inclusions of the cell. The four main areas, capsule, cell wall, cytoplasm and polyphosphate bodies (PPB's) were analyzed using x-ray analysis. For each heavy metal to which the cells were exposed, twenty random cells were analyzed. In addition a control group of *Rhodotorula rubra* cells was also analyzed. Figure 94 is an example of a typical x-ray or EDAX spectrum of *the cell wall a Rhodotorula rubra* cell exposed to 20 ppm of Al. It shows the presence of C, O, S, K, P, a range of major elements that are characteristic of this species. Al is also evident by the large peak in the spectrum. The heights of the peaks are not indicative of the amounts of the elements contained in the particular body of the organism. In each case the control showed no trace of the test metal.

Figure 3 shows the mean results of the quantitative analysis done on the EDAX spectrum of twenty test samples of the *Rhodotorula rubra*. I report

here only the amounts of the particular heavy metal that the cells were exposed to. These results were obtained by performing quantitative analysis on each individual spectra, the mean results are shown in Table 13, please keep in mind that the results are reported in percentages; parts per million or ppm are equivalent to the values under the column header "Norm Wt.%"(Fig.13-14).

The results from the quantitative analysis of the different cellular components ie.capsule, cell wall, cytoplasm, and polyphosphate body, were analyzed using a combination of Statview 4 and JMP 4. The results 95-101 and the results of the one-way ANOVA. JMP 4 program was used to generate a MANOVA and scatter plots, found in Appendix E. In addition all the numbers reported in Table 1 were then used to calculate the final volumes reported in Table 13.This was obtained by using the appropriate formula for the shape of the organism. All of this data including the data in Table 2 clearly show that for all the metals including Cd the polyphosphate body averaged a higher uptake of the metal the cell was exposed to then do the other inclusions in the cell.

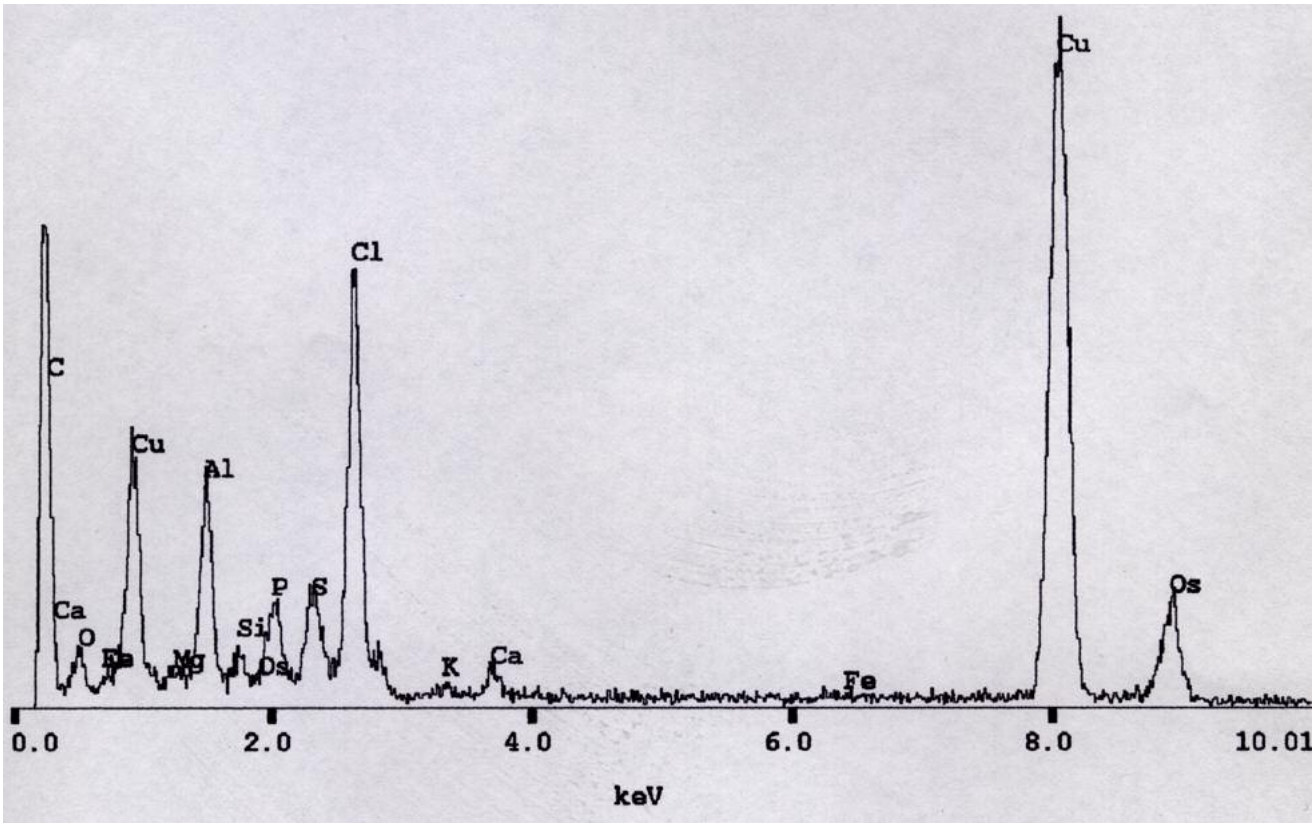



Figure 94: EDAX spectrum of the cell wall of *Rhodotorola rubra,* exposed to 20 ppm of Al. Notice peaks of C, S, and Cl.

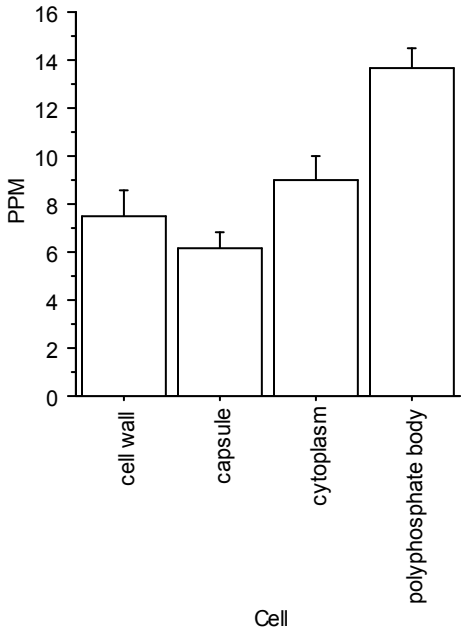



Figure 95: Bar graph for *Rhodotorola rubra* exposed to Al. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=11.264$; 3, xdf; $p<0.0001$).

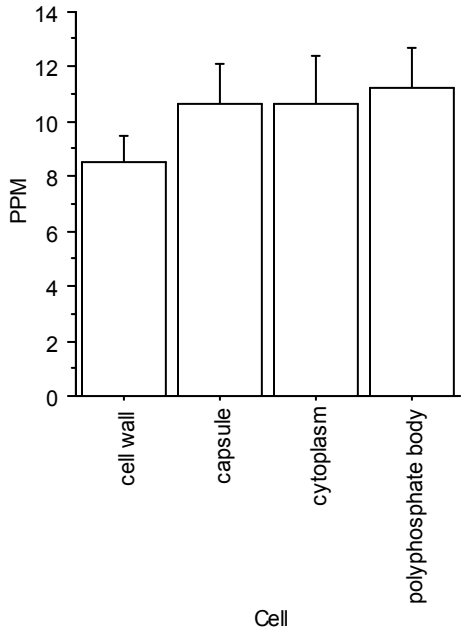



Figure 96: Bar graph for *Rhodotorola rubra* exposed to Cd. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=13.047$; 3, xdf; $p<0.0001$).

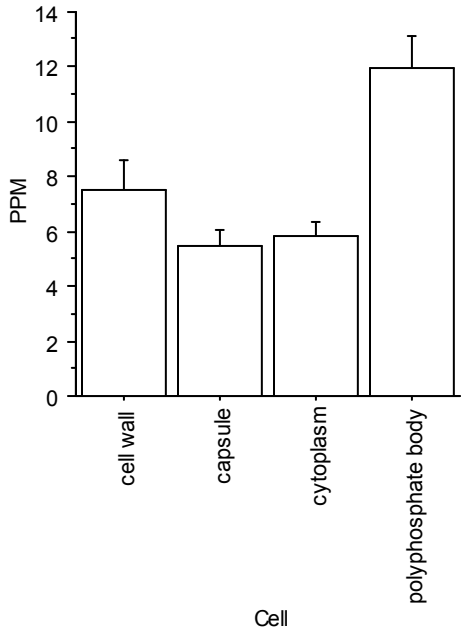



Figure 97: Bar graph for *Rhodotorola rubra* exposed to Cu. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=13.047$; 3, xdf; $p<0.0001$).

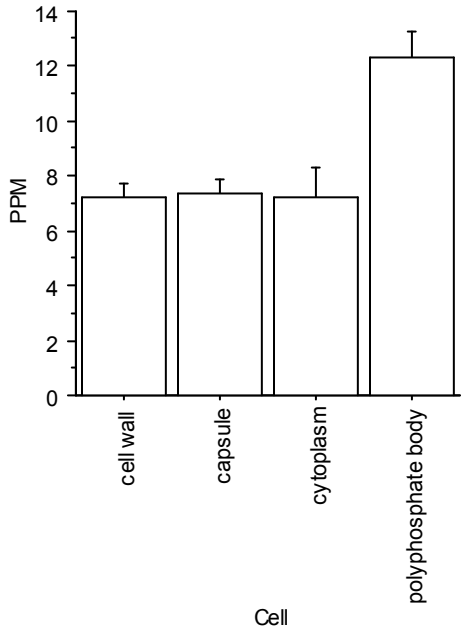



Figure 98: Bar graph for *Rhodotorola rubra* exposed to Mn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=13.047$; 3, xdf; $p<0.0001$).

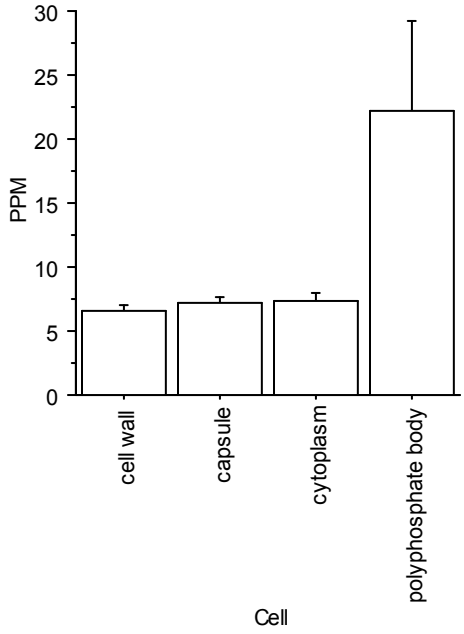



Figure 99: Bar graph for *Rhodotorola rubra* exposed to Ni. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=13.047$; 3, xdf; $p<0.0001$).

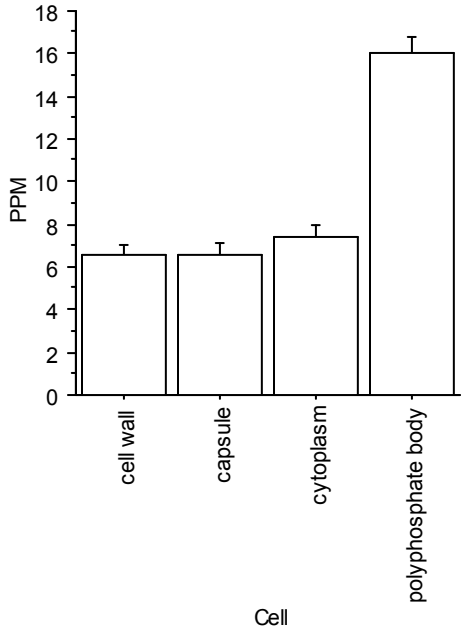



Figure 100: Bar graph for *Rhodotorola rubra* exposed to Pb. The numbers for the cell means are given in PPM's. ±1SE. The means are significant in ANOVA ($F=13.047$; 3, xdf; $p<0.0001$).

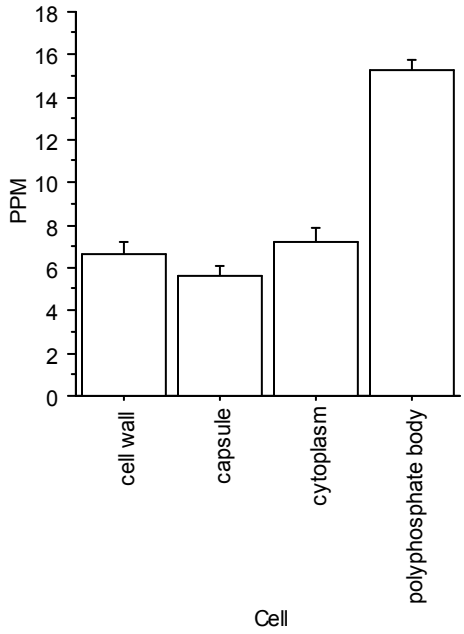



Figure 101: Bar graph for *Rhodotorola rubra* exposed to Zn. The numbers for the cell means are given in PPMs. ±1SE. The means are significant in ANOVA ($F=13.047$; 3, xdf; $p<0.0001$).

DISCUSSION

When we look into a basic introductory biology textbook we see notes detailing the breakdown of the Kingdom Monera into two major groups: the archaebacteria and the eubacteria (Alberts *et al*.1989) For many years the archaebacteria nearly escaped detailed study due to the difficult nature of their cultivation. These prokaryotes which can be distinguished on the basis of their cell wall and plasma membrane include the oceanic methanogens as well as the species which have adapted to more extreme environments, the halophiles and the thermoacidophiles. The cell wall of these species generally lacks a murein or pseudomurein layer.

The more advanced of the prokaryotes, the eubacteria, include the rickettsiae, chlamydias, and mycoplasmas along with the more familiar photosynthetic species such as the cyanobacteria. The eubacteria are separated into two major and one minor group. These are based on the nature of their cell walls, gram positive describes those cells that take up the stain upon exposure, and gram negative those cells that do not. Some cells

will stain either positive or negative depending on whether certain conditions, like growth phase or nutritional status and are actually called gram variable (Beveridge, 1989). The cyanobacteria closely resemble the negative bacteria with peptidoglycan as the principal wall component. Peptidoglycan is reported to have a metal-binding capacity (Beveridge et al., 1982).

Microscopy shows the cyanobacterial wall to be composed of four layers that are numbered from the cell's inside as LI, L II, L III, and L IV (Sicko-Goad and Jensen, 1971). In addition to peptidoglycan this wall is structured of simple sugars, muramic and glutamic acids, diaminnopimelic acid, galactosamine, glucosamine and alanine (Trainor, 1978).

The negative charge on the cell wall and plasma membrane results from carboxyl groups, phosphate groups of membrane phopholipids, and amino acid residues of membrane proteins. These groups are the source of oxygen, nitrogen and sulfur for which various cations will have affinities (Nieboer and Richardson, 1980; Remacle, 1990).

Previously, several groups have demonstrated that prokaryotes, and even some eukaryotes, will bioconcentrate certain heavy metals in their inclusions/organelles, especially their polyphosphate bodies (Jensen et al.1982a, 1982b, 1986). The varying habitat range and the influence of the surrounding organic material of algae, in addition to their potential or protein production, waste treatment and chemical production (Grobbelaar, 1988) merit this group a precedence in future scientific study. As part of the aquatic ecosystems, algae are threatened by increasing levels of pollution that cause greater levels of acidification. In the fact that algae exhibit a greater sensitivity to environmental variation strengthens the rationalization for using them as a test species in order to provide greater environmental protection (Hörnström, 1990). As photosynthetic, oxygen-yielding organisms, the cyanobacteria become primary producers, the first link of the food chain, or the initiators of the energy transfer system in an ecosystem. These organisms even on their own are worthy of scientific study but they become more important when we take into consideration the theory that pollutants tend to accumulate as they move up the food chain. The case of increase in the residual concentrations of DDT in a Long Island, New York, salt marsh highlights the severity of the accumulation. In this study, plankton showed a total residue of 0.04ppm of the deadly chemical (Laws 1981).

These concentrations multiplied through the minnows and predatory fish up to the cormorant with 26.4ppm,an increase of 660 times that found among plankton. The results can be used to predict and to compare the effects of pollutants on organisms at higher trophic levels.

Intracellular metal sequestering has been reported in a number of studies, involving different microorganisms. Jensen and his co-workers have investigated the heavy metal binding of polyphosphate bodies in laboratory cultures of microbes. They have shown that these bodies bind significant amounts of Ba, Mg, Mn, Cd, Co, Cu, Hg, Ni, Pb, and Zn. Baxter and Jensen, 1980a; Jensen et.al.,1982a; Jensen et al. 1982b Rachlin et al. 1984; Jensen1986, Kunst and Roomans 1985 have shown that Cr, Ni, and Cd were also sequestered in polyphosphate bodies. Sicko-Goad and Stoermer (1979) showed that Pb and Cu were sequestered in *Diatoma temie* var. *elongatum.* Similar results were reported for *Plectonema* and *Schizorthirx* species exposed to Zn. Sequestering of heavy metal in the cytoplasm of certain prokaryotes has been shown to occur. Jensen and co-workers have shown that for cyanobacteria such as *Plectonema boryanum* a small amount of Cd, Ca, Pb, and Zn appeared in the cytoplasm.

Microbes have evolved a number of ways to tolerate environments, which are rich in heavy metals. These characteristics have allowed them to survive while organisms that lack these adaptive properties cannot grow in these niches. Numerous investigators have carried out studies of metal uptake by microbes. It appears that metal exposed to any organism will be sequestered if the organism does not have an exclusion mechanism. Many microbes possess plasmids that confer on them resistance to a heavy metal.

A number of studies have suggested that the capsule or sheath of the cell is the main sequestering site. Horikoshi et al. (1981) studied uranium accumulation in 10 Gram-negative bacteria, 13 actinomycete, 11 yeasts and 18 additional fungi. Two of the actinomycetes accumulated uranium in significant amounts: *Actinomyces levoris* and *Streptomyces viridochromogenes* no. 19. EDTA released the metal, indicating surface binding, probably capsular. Karapanagiotis et al. (1990) have shown that bacterial exopolymer and fulvic acid extracted from a sewage plant bind Cu,

Cd, Ni, and Zn. Dune and Bull (1983) have shown that Cu, will bind to walls of bacteria in activated sludge. Loaec et al. (1997) extracted exopolysaccharide from *Alteromonas madeodii* and found that it bound Pb, Cd, and Zn.

In addition to phosphorus a number of other components have been reported to make up the PPB's. Widra (1959) reported that the polyphosphate bodies of *Aerobacter aerogenes* were composed of phosphorus, magnesium, lipid, and RNR. Rosenberg (1966) and Munk and Rosenberg (1969) isolated the polyphosphate bodies from *Tetrahymena pyriformis* and found that they consist of calcium magnesium and pyrophosphate.

Polyphosphate bodies are found to be common cytoplasmic inclusions in microorganisms, such as bacteria, algae, and fungi. Jensen (1968; 1969) modified a lead sulfide-staining procedure, which was reported to be specific for PPB's (Ebel et al., 1958) and demonstrated which cellular inclusion in PPB's under the transmission electron microscope. Under the electron beam, the PPB's were identified as electron dense, spherical and sometimes porous

granules (Jensen, 1990). They, as a result of the dense nature, often fall out or get chipped out in sectioning (Jensen, 1968) The bodies can also be seen in living cells by phase contrast microscopy. PPB's in cells are visualized by the TEM, this combined with EDX analysis, allow us to detect the phosphorus Doonan et al., 1979; Baxter and Jensen, 1980a).

The studies on heavy metals in our laboratory began in about 1988, before I entered the laboratory. At that time we hypothesized based on our studies of microbial polyphosphate bodies (PPBs) that they would probably sequester heavy metals problematic to the environment. We first had to develop a methodology by which we would be able to obtain data in this regard. We succeeded in setting up our scanning electron microscope with a scanning transmission mode with an associated energy dispersive spectrometer. With a lot of manipulation it was possible to view and obtain spectra from these bodies in air-dried cells (Baxter and Jensen, 1980b). We went on with this system to demonstrate that the PPBs do indeed sequester, in seemingly large amounts most metals to which we exposed cells (Baxter and Jensen, 1980a; Jensen et al., 1982a; Jensen et al., 1982b). Our approach at this time developed into a three-pronged approach. We exposed cells to a metal and

determined their $EC_{50}$. This is the amount of the metal that reduces the growth of the organism by 50%. We then exposed cells to one log of the metal above and below this amount. From thin sections we then carried out morphometric analysis to determine what affect the metal was having, if any, on the structure of the cell. Our third determination was to ascertain where in the cell the metal might have been sequestered. For this we used our SEM operating in the STEM-spot mode. We were able to determine if the metal was mainly in the capsule (sheath), cell wall, cytoplasm or PPBs. The resolution of our first system was not the best and the system did only qualitative analysis. Using this approach we were able to determine the level at which a number of heavy metals become a problem for growth, what cellular inclusions the metal may target and where the metal has been sequestered in the cell. Our present system as discussed in Methods is the "state of the art" system. Since the work of Hall (Hall et al., 1973; Hall, 1989; Roomans, 1990) it became evident that high accelerating voltage in the electron microscope produced a much better result than low accelerating voltages for carrying out quantitative analysis. The methodology, therefore, shifted to TEMs rather than SEMs.

The first research project using this new system was to determine the weight of PPBs in several microbes, *Plectonema boryanum, Staphylococcus aureus* and several bacteria from Lake Arthur in Black Rock Forest, New York. This was also the first research project thet I was involved with in the laboratory.

With the advent of new technology and the contributions made by Hall and Heldal it is now therefore possible to determine not just percent of the total elements which are in the probed area, but also the amount in grams or smaller units. I can thus determine which cell area or inclusion has sequestered a metal and then the amount contained therein. We can then extrapolate these data to the whole cell by using simple mathematics. We can calculate the volume of the capsule, cell wall, cytoplasm and PPBs. This will give the data we need to determine which cell component sequesters a metal in the largest amount.

Overall the data will have application in a number of biological areas including health. Heavy metals in water have been shown to be a problem in regard to human health. Some are known carcinogens while others most

likely do cell damage to specific cell inclusions as well as interfering with the normal biochemical reactions in cells.

In this comprehensive study on the uptake of different metals by the four different inclusions found in a cell. The results reported in this research indicate that whereas there is uptake of metal by the four components of the cells, the capsule, cell wall, cytoplasm and the polyphosphate bodies, as a whole the polyphosphate bodies uptake much more metal on average. This is evident when we look at the numbers in Tables 3-13 and compare the amounts of metal in $\mu m^3$ sequestered in each inclusion/organell. Each PPB can take up on an average between 5.6 ppm and 19.9ppm of the metals regardless of which metal it is.

# APPENDICES

## Appendix A

Composition of Culture Medium-Modified Fitzgerald (Fitzgerald *et al*., 1952)

| | mg/liter |
|---|---|
| $NaNO_3$ | 124 |
| $K_2HPO_4 \bullet 3H_2O$ | 13 |
| $MgSO_4 \bullet 7H_2O$ | 25 |
| $CaCl_2 \bullet 2H_2O$ | 36 |
| $Na_2CO_3$ | 20 |
| $NaSiO_3 \bullet 9H_2O$ | 58 |
| Ferric Citrate | 3 |
| Citric Acid | 3 |
| Gaffron's minor elements solution | 0.04 ml. |

pH is adjusted to 7.2 by adding 1n HCl or 1N NaOH

Gaffron's solution

| | g/liter |
|---|---|
| $H_3BO_3$ | 3.10 |
| $MgSO_4 \bullet 4H_2O$ | 2.23 |
| $ZnSO_4 \bullet 7H_20$ | 0.287 |
| $(NH_4)_6 Mo7O_{24} \bullet 4H_2O$ | 0.088 |
| $CuSO_4 \bullet 5H_2O$ | 0.125 |
| $Co(NO_3)_2 \bullet 6H_2O$ | 0.146 |
| $Al2(SO_4)_3KSO_4 \bullet 24H_2O$ | 0.474 |
| $NiSO_4(NH_4)SO_4 \bullet 24H_2O$ | 0.474 |
| $Cd(NO_3)_2 \bullet 4H_2O$ | 0.154 |
| $Cr(NO_3)_3 \bullet 7H_20$ | 0.037 |
| $V_2O_4(SO_4)_3 \bullet 16H_20$ | 0.035 |
| $Na_2WO_4 \bullet 24H_20$ | 0.033 |

| | |
|---|---|
| KBr | 0.119 |
| KI | 0.083 |

Appendix B

Fixation of experimental samples.

A. Preparation of solutions:

Stock Michaelis Buffer:

a. Weight: 1.94 g sodium acetate ($NaC_2H_3O_2 \bullet 3H_20$)

2.94 g sodium barbital

3.40 g sodium chloride

b. Add water to (a.) to reach 100 ml and dissolve the chemicals, then store the buffer is a refrigerator.

1. Osmium Tetraoxide:

A stock solution of 2% $OsO_4$ was made by dissolving 1g of osmium tetraoxide in 50 ml of distilled water. The solution was stored in a Pyrex glass stoppered bottle, which was placed in a tin can, and stored in a refrigerator.

2. 1% Bacto-tryptone:

1 g of bacto-tryptone and 0.5 g NaCl were dissolved in 100 ml distilled water. The solution was place in small closed vials and stored frozen. The contents of the vials were thawed and mixed thoroughly before use.

B. Fixing procedure of modified Kellenberger *et al*., 1958; Pankratz and Bowen,1963):

1. Preparation of solutions:

a. 5 ml Stock Michaelis Buffer,

7 ml 0.1 N HCl,

13 ml distilled water

0.25 ml 1.0 M $CaCl_2$;

then adjust the solution to pH 6.1 or 6.2.

b. Dilute solution (a) with an equal amount (1:1) of 2% $OsO_2$ then add 0.1 ml 1% bacto-tryptone per ml.

2. Fix samples in solution (b) for 3 hours at room temperature.

Appendix C

Embedding with Epon 812 (Luft, 1961)

A. Preparation of Epon:

1. Mixture A:

62 ml Epon 812,

100 ml DDSA (Dodenyl Succinic Anhydride).

2. Mixture B:

100 ml Epon 812,

89 ml NMA (Nadic Methyl Anhydride).

Mixture A and Mixture B were refrigerated, and brought to room temperature before opening to avoid water condensation.

2. Epon mixture for embedment: 1 part A, and 2 parts mixture B were mixed in as disposable plastic beaker, and 0.2ml of DMP-30 was added per 10 ml of the epon mixture. It is important that all components are thoroughly mixed so that no "streaks" are visible. Epon mixture should be freshly made before embedding.

B. Dehydration and embedding of fixed samples:

| | |
|---|---|
| 50% ethanol | 5-10 min |
| 75% ethanol | 5-10 min |
| 95% ethanol | 5-10 min |
| 100% ethanol | 5-10 min |
| 100% ethanol | 5-10 min |
| propylene oxide | 5-10 min |
| propylene oxide | 5-10 min |
| propylene oxide | 5-10 min |
| 3 propylene oxide : 1 epon | 15 min |
| 1 propylene oxide : 1 epon | 30 min |
| 1 propylene oxide : 3 epon | 60 min |

Transfer the samples to Beem capsules then fill with pure Epon. Polymerize the Epon at:

| | |
|---|---|
| Room temperature | overnight |
| 35° C oven | 8-24 hours |
| 45° C oven | 8-24 hours |
| 60° C oven | 8-24 hours |

Appendix D

Uranyl acetate-methanol stain (Stempak And Ward, 1964).

A: Preparation of the solution: 15g hydrated uranyl acetate ($UO_2(CH_3COO)_2 \bullet 2H_2O$) were dissolved in 50 ml of acetone-free absolute methanol with a magnetic stirrer . The solution was then stored in a Pyrex glass- stoppered bottle at 4 °C.

B: Staining: Grids were immersed in wells of a porcelain staining dish, section side up, for 10 minutes at room temperature. The wells were covered during this period to prevent evaporation of the methanol.

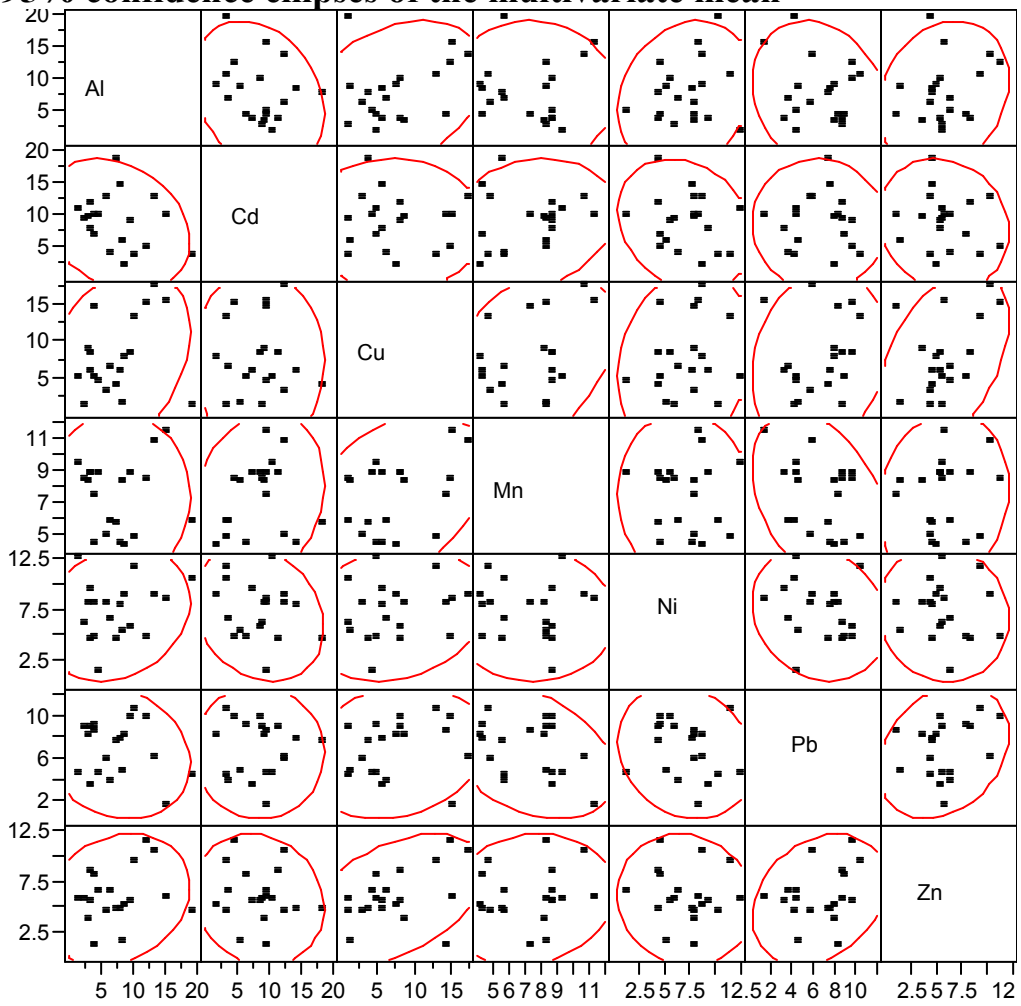
C. Washing: The grids were then rinsed with absolute methanol, ethanol and water as listed below:

| | | |
|---|---|---|
| 1 | Absolute methanol | 2-3 dips |
| 2 | Absolute methanol | 10-20 dips |
| 3 | 100% ethanol | 25 dips |
| 4 | 80% ethanol | 50 dips |

| | | |
|---|---|---|
| 5 | 50% ethanol | 50 dips |
| 6 | distilled water | 50 dips |

Appendix E: Final numbers on percentages of metal found in the different components of the cell.

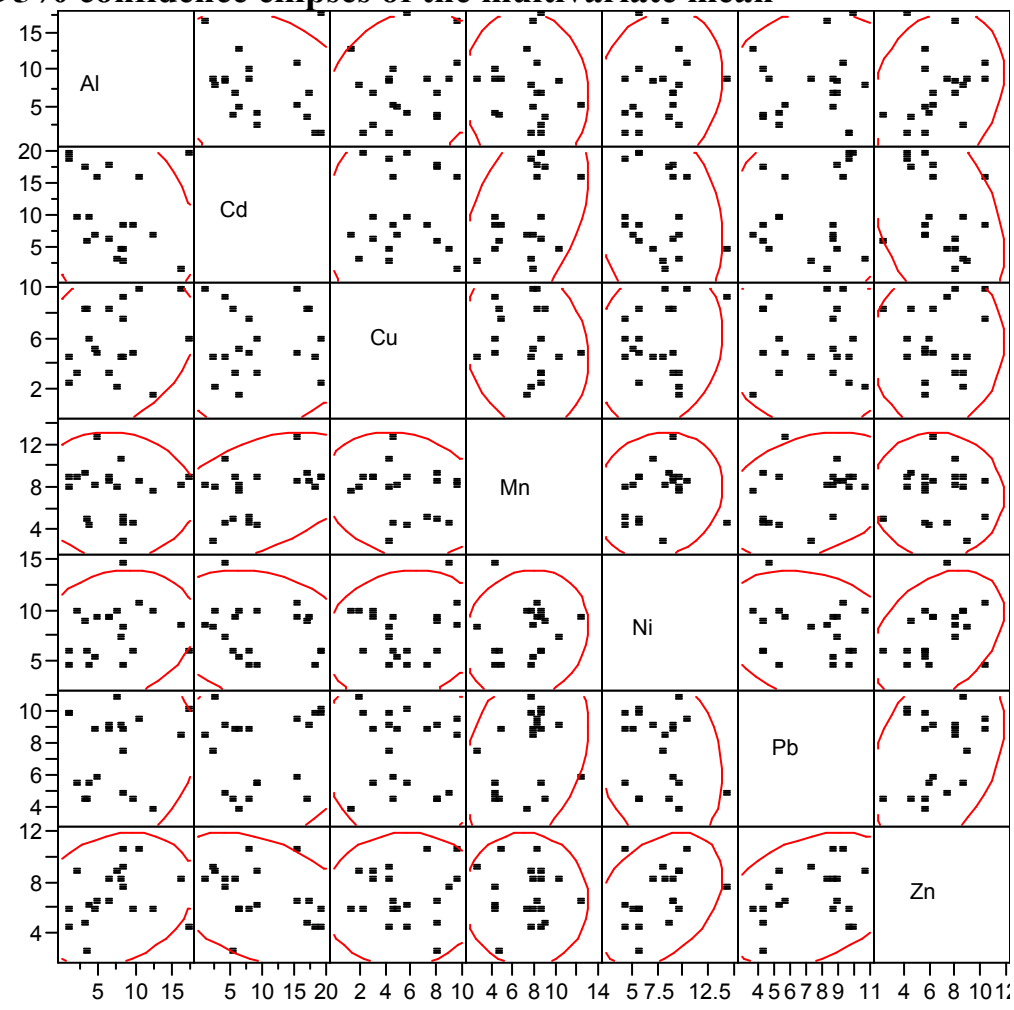
***Plectonema boryanum***
**Capsule**
**95% confidence ellipses of the multivariate mean**




Wilk's Lambda=1,F=14 22,202df

**Cell Wall**

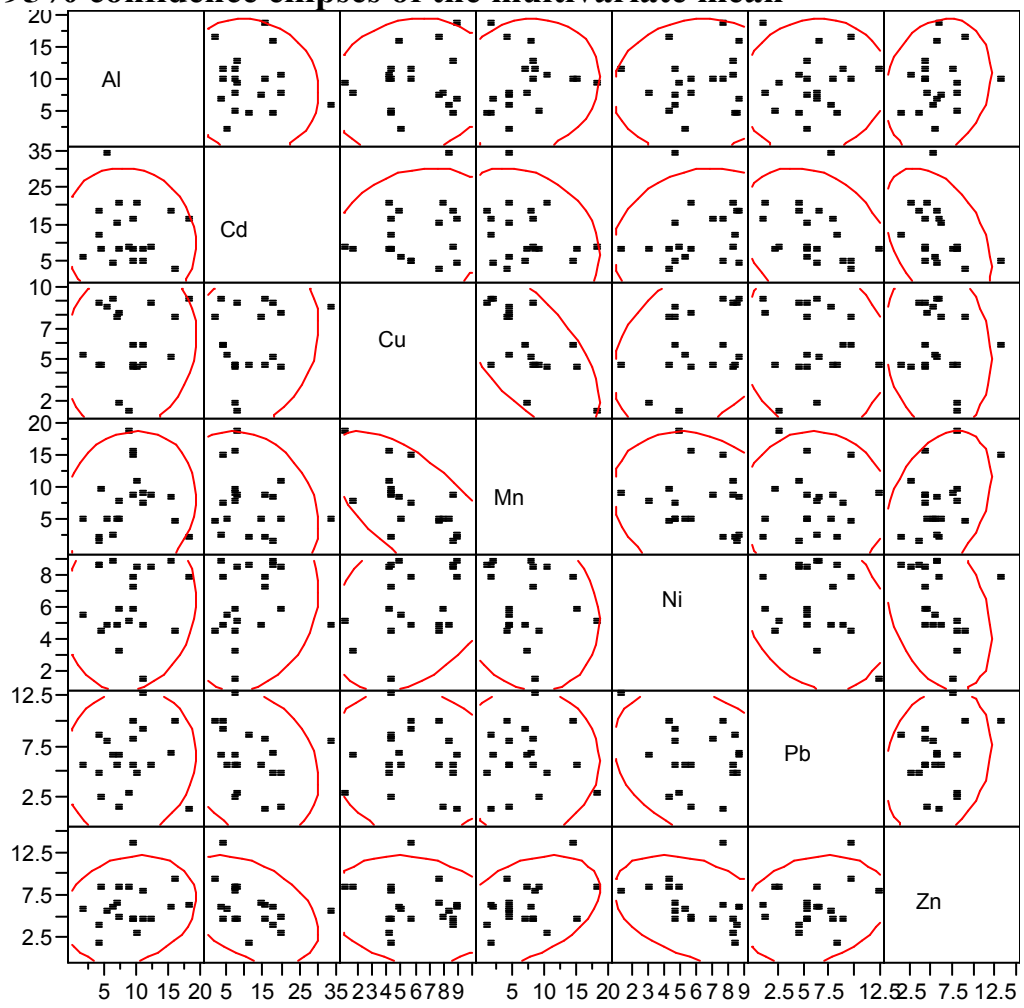
**95% confidence ellipses of the multivariate mean**




Wilk's Lambda=1,F=1.4 20,202df

**Cytoplasm**

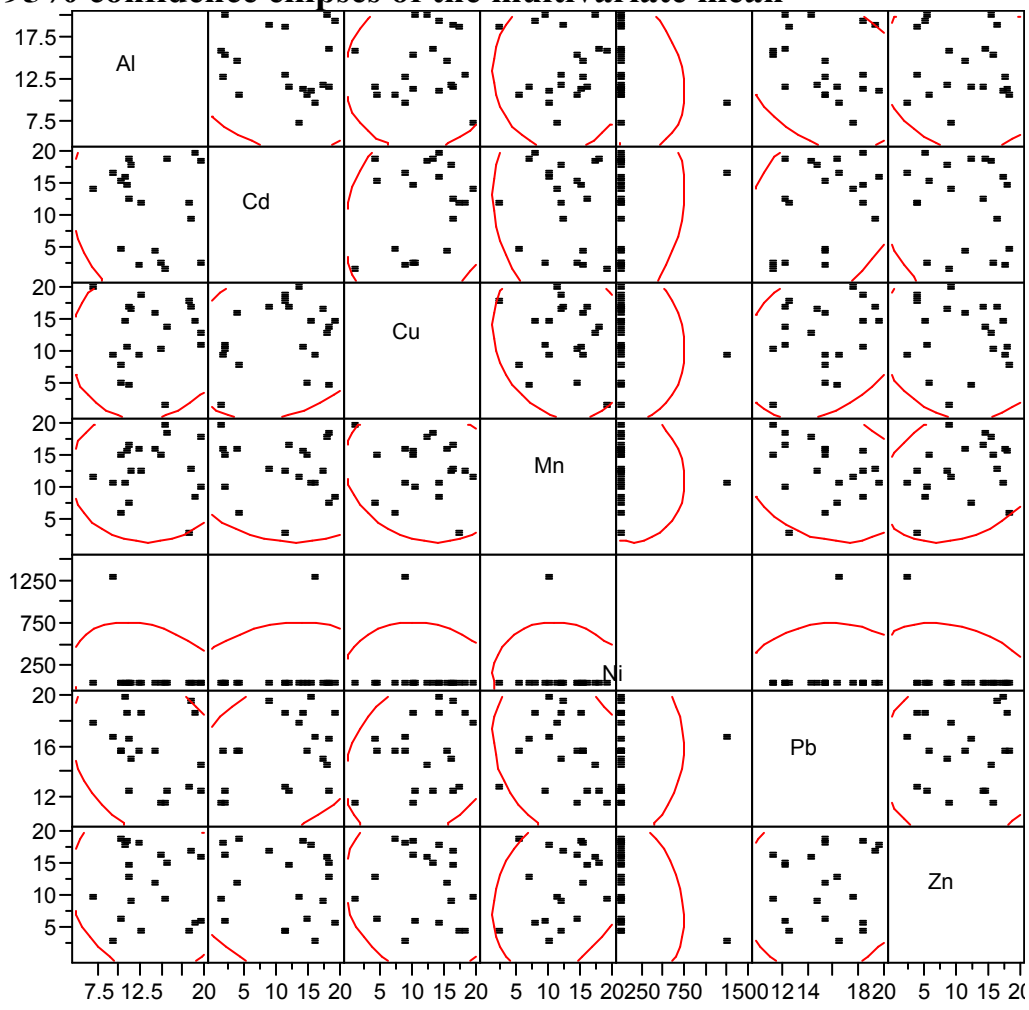
**95% confidence ellipses of the multivariate mean**




Wilk's Lambda=1,F=14.4 29,608df

**PPB**

**95% confidence ellipses of the multivariate mean**

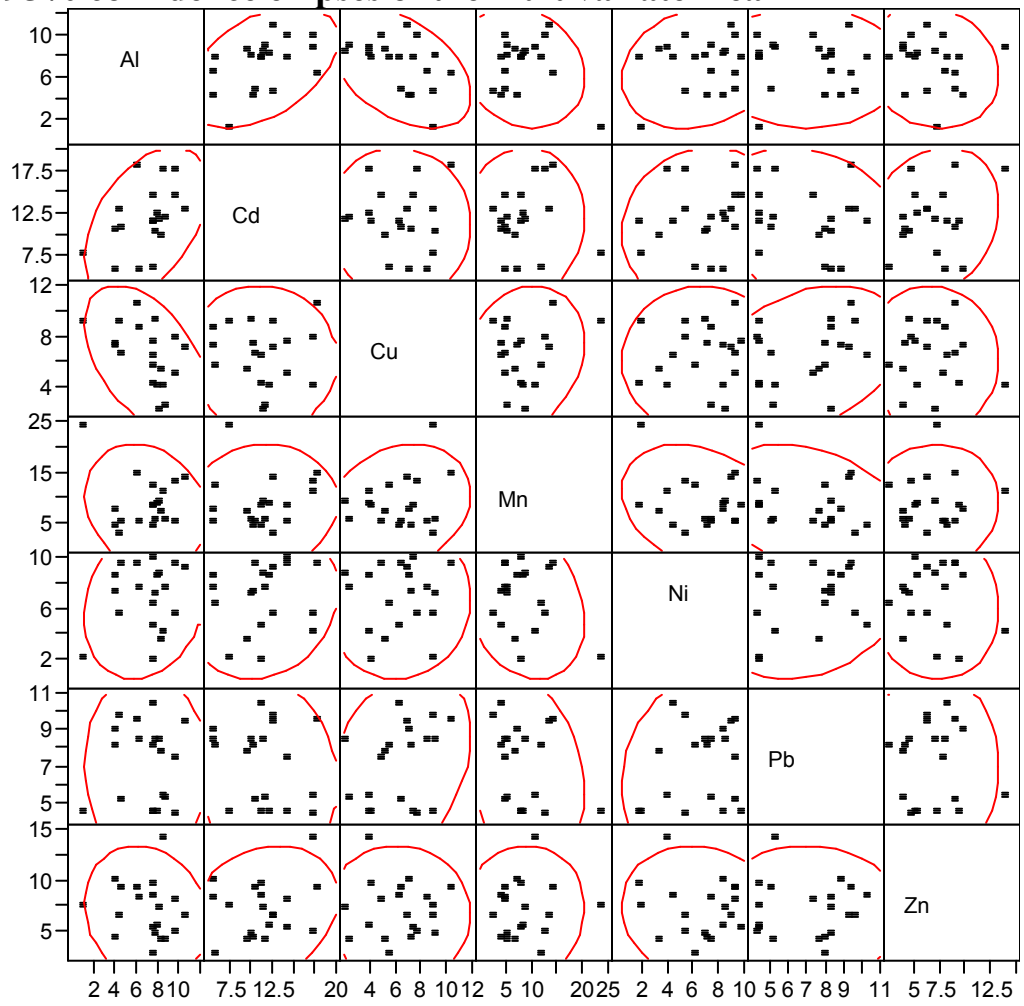



Wilk's Lambda=1, F=74.4  29,608df

*Arthrobacter globiformis*

**Capsule**

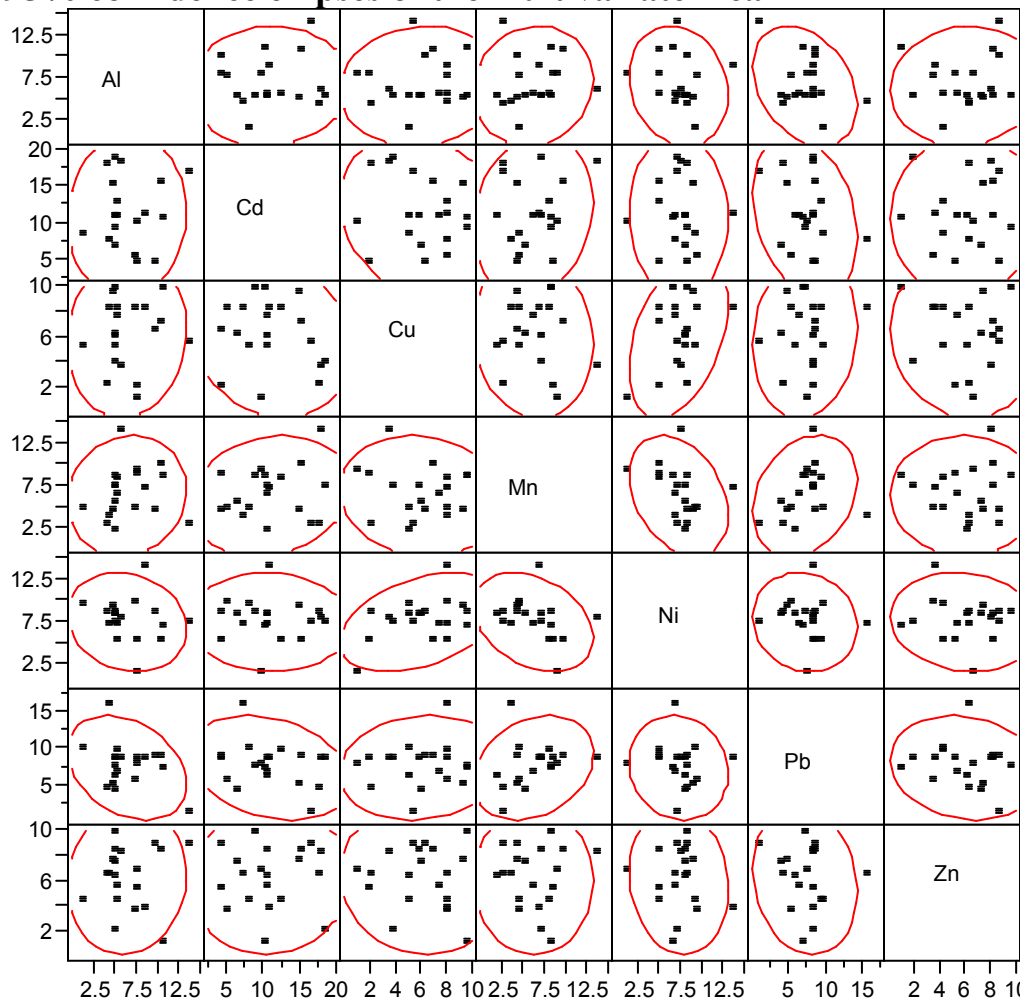
**95% confidence ellipses of the multivariate mean**




Wilk's Lambda=1,F=8.34 14,008df

## Cell Wall

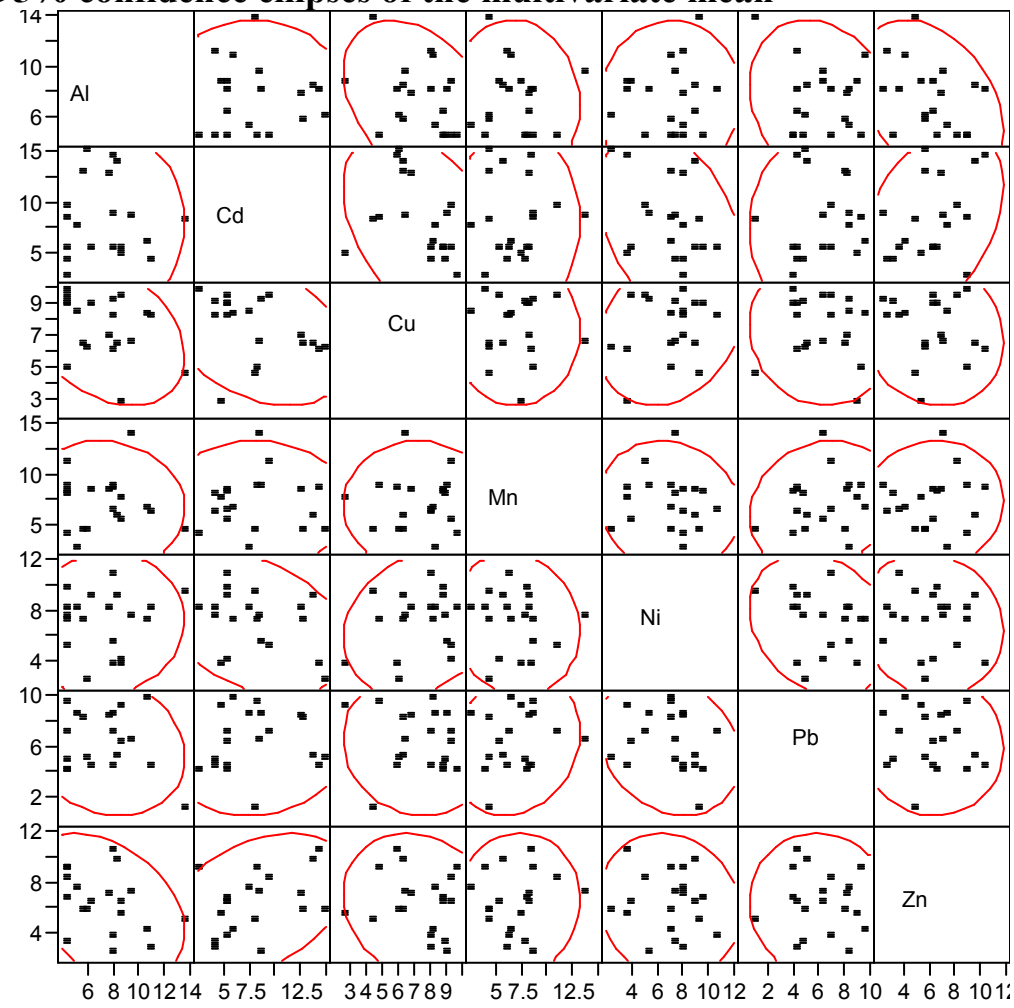
**95% confidence ellipses of the multivariate mean**




Wilk's Lambda=1,F=3.78  14,006df

## Cytoplasm

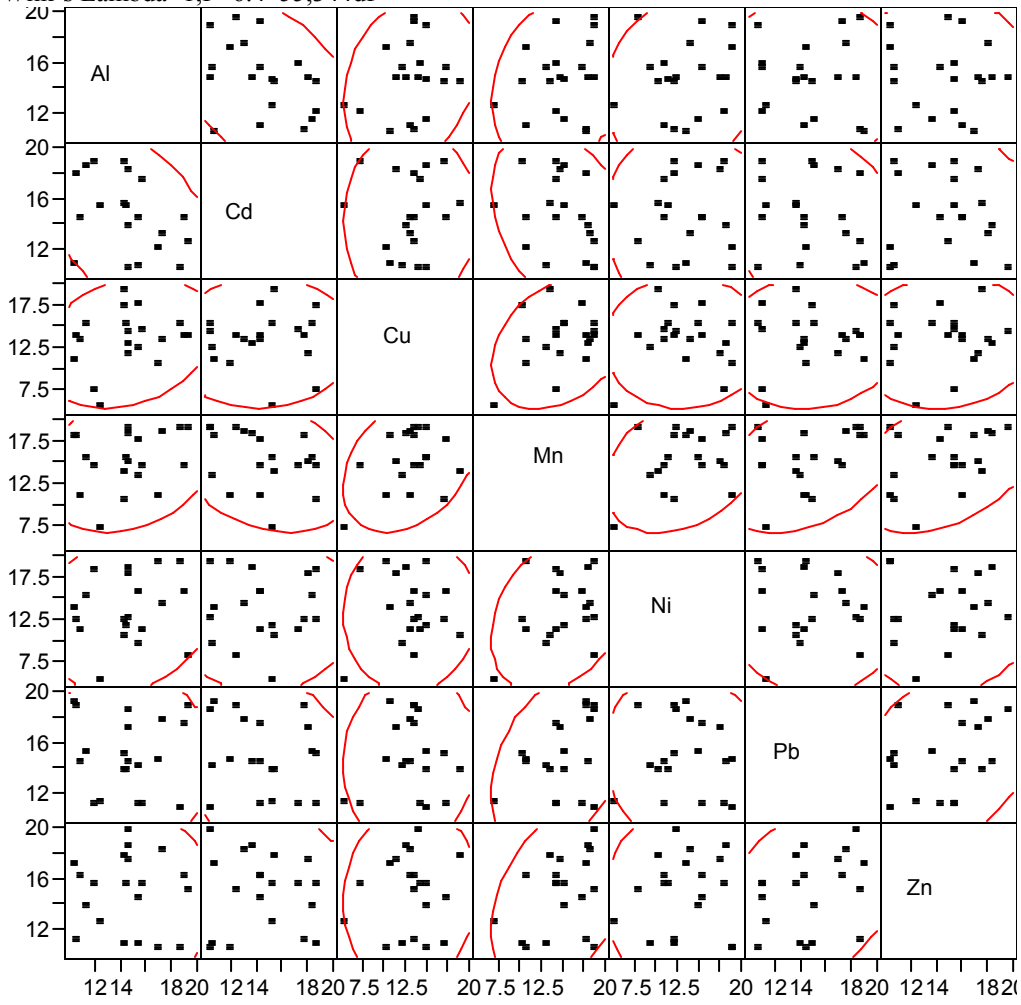
**95% confidence ellipses of the multivariate mean**




Wilk's Lambda=1,F=14.4  29,608df

## PPB **95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=6.4  55,344df

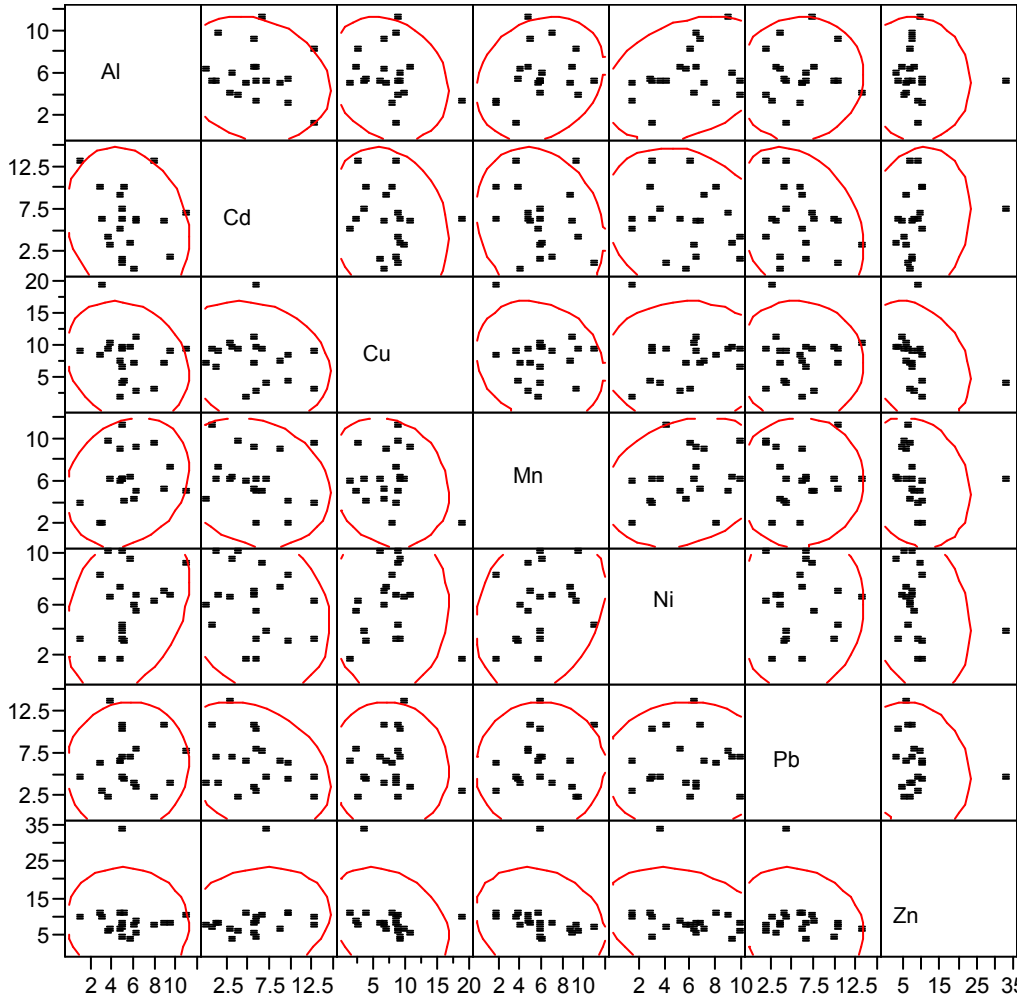


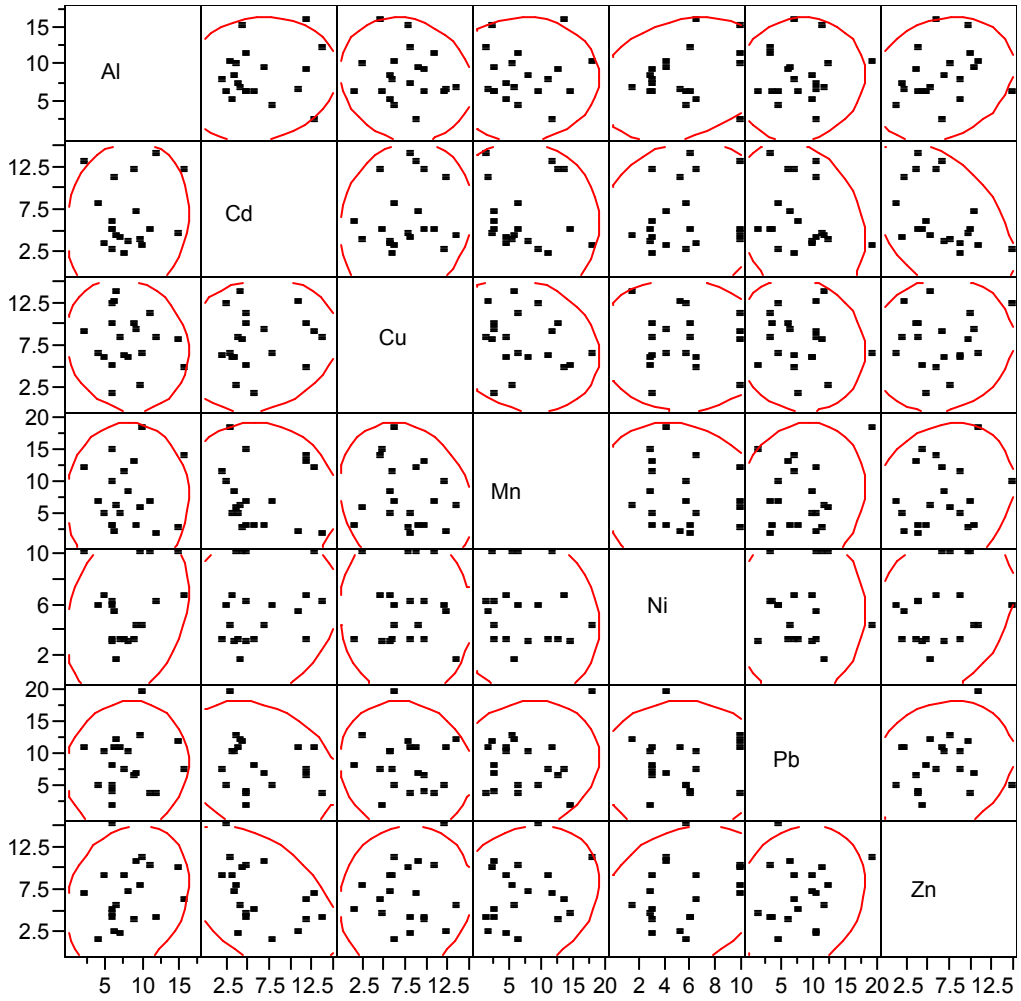
*Gloeocapsa alpicola*

**Capsule**

**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=6.4  55,344df



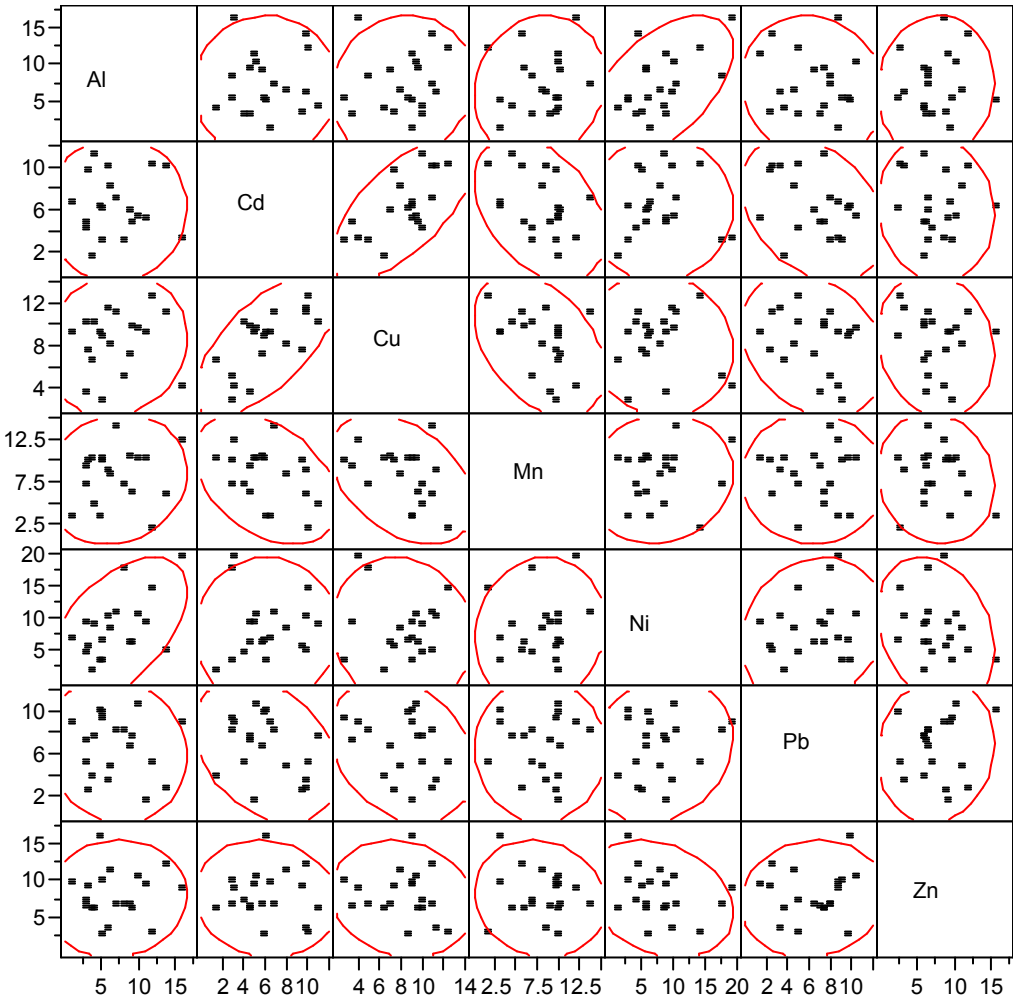



**Cell Wall**

**95% confidence ellipses of the multivariate mean**

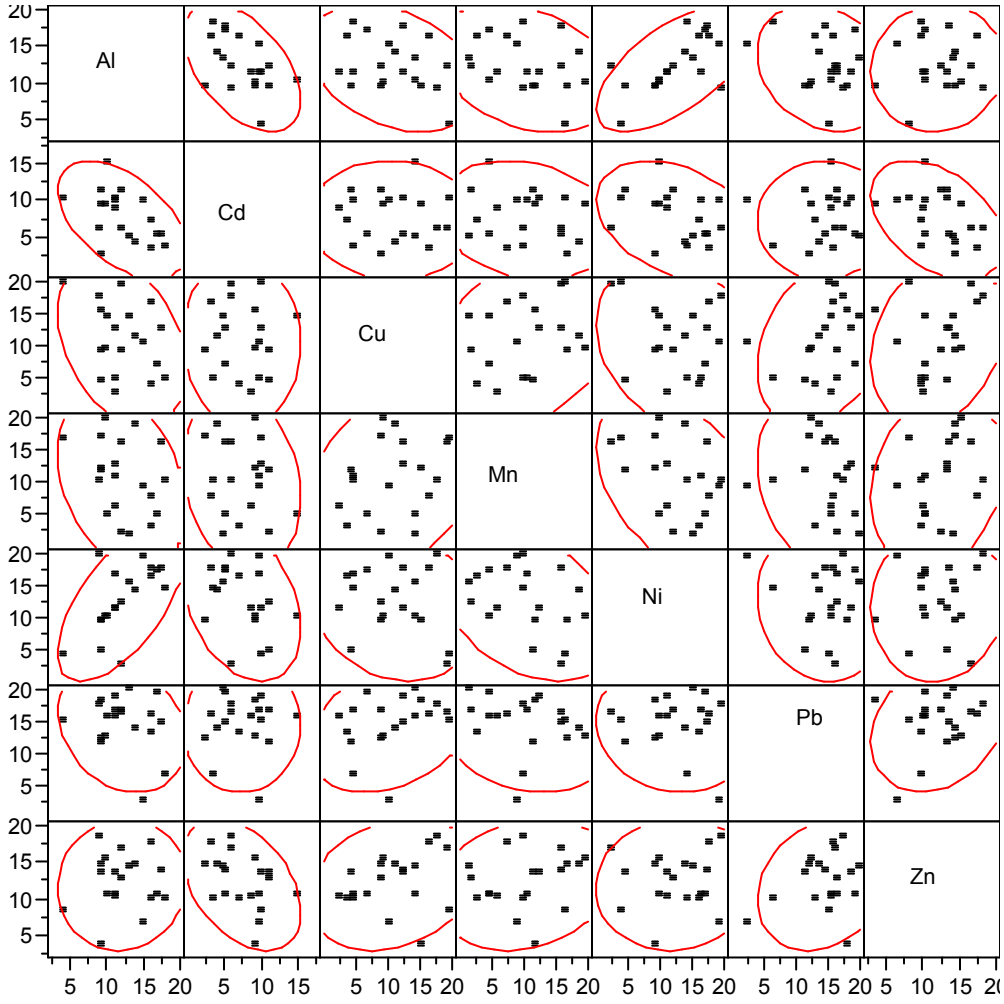
Wilk's Lambda=1,F=6.4  55,344df

**Cytoplasm**
**95% confidence ellipses of the multivariate mean**

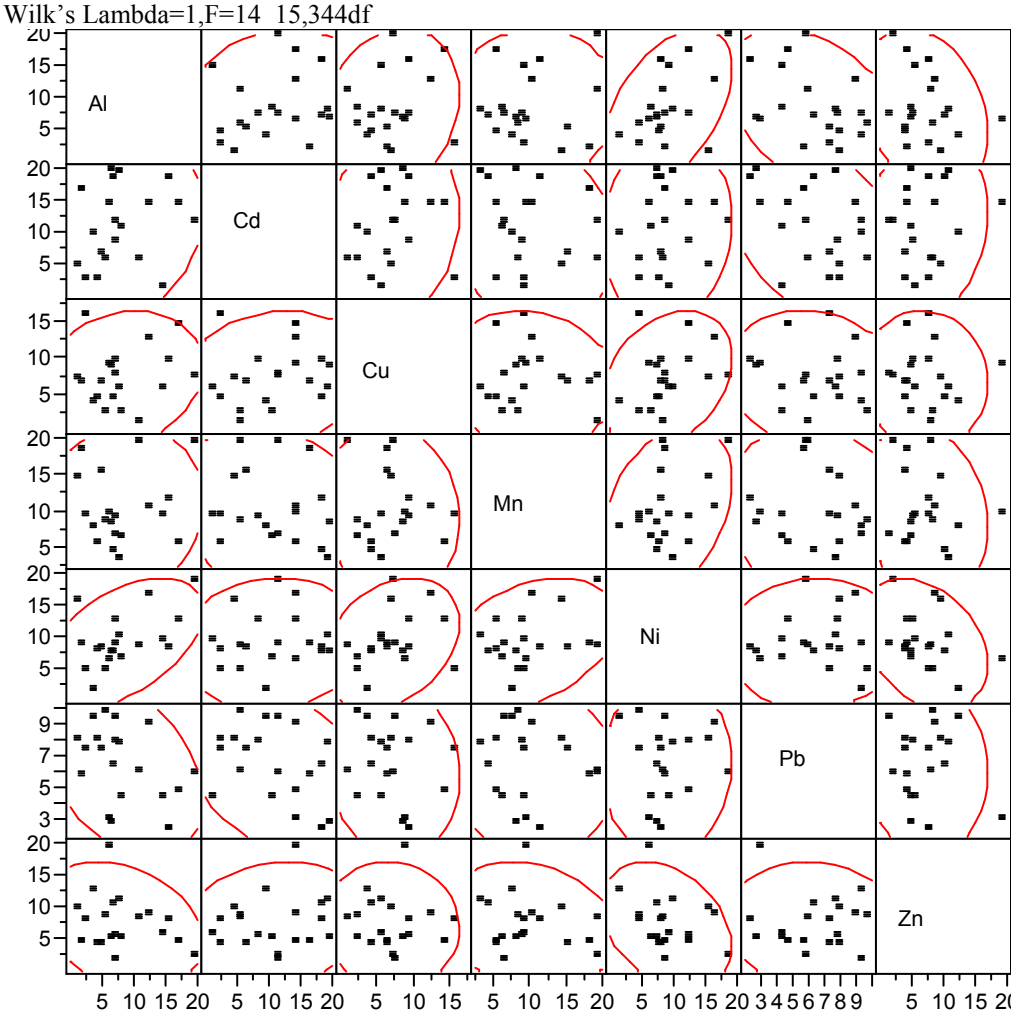


**PPB**

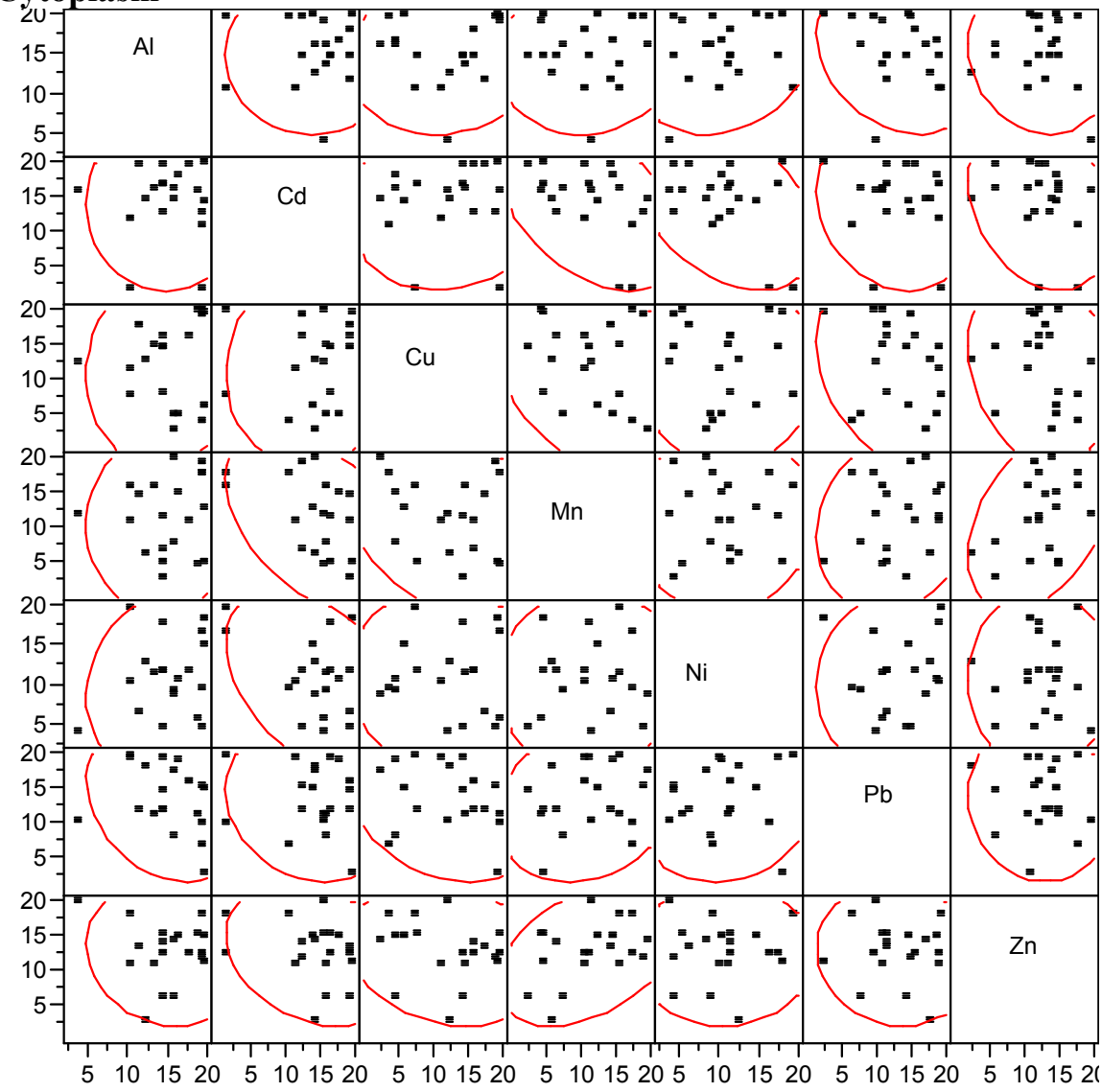
**95% confidence ellipses of the multivariate mean**



***Synechococcus leopoliensis***
**Cell Wall**
**95% confidence ellipses of the multivariate mean**
Wilk's Lambda=1,F=14 15,344df



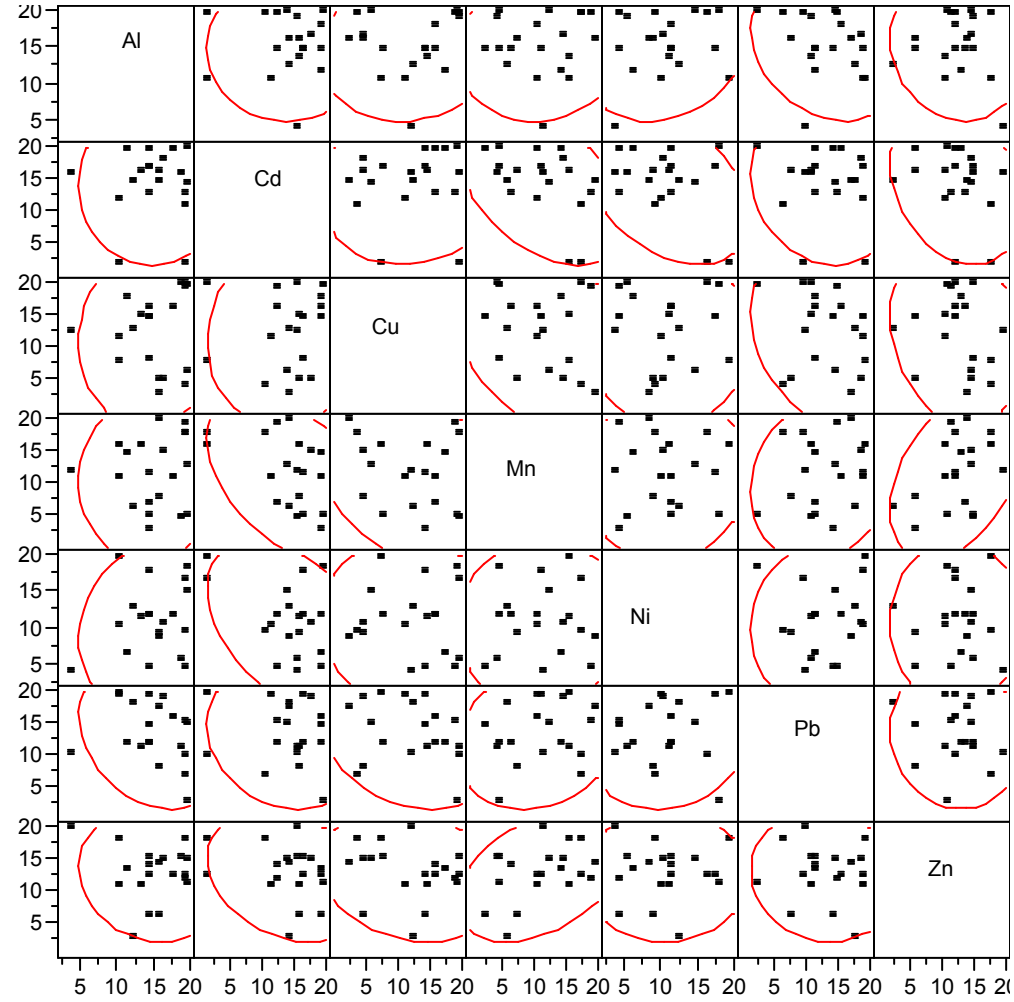
**Cytoplasm**




**95% confidence ellipses of the multivariate mean**

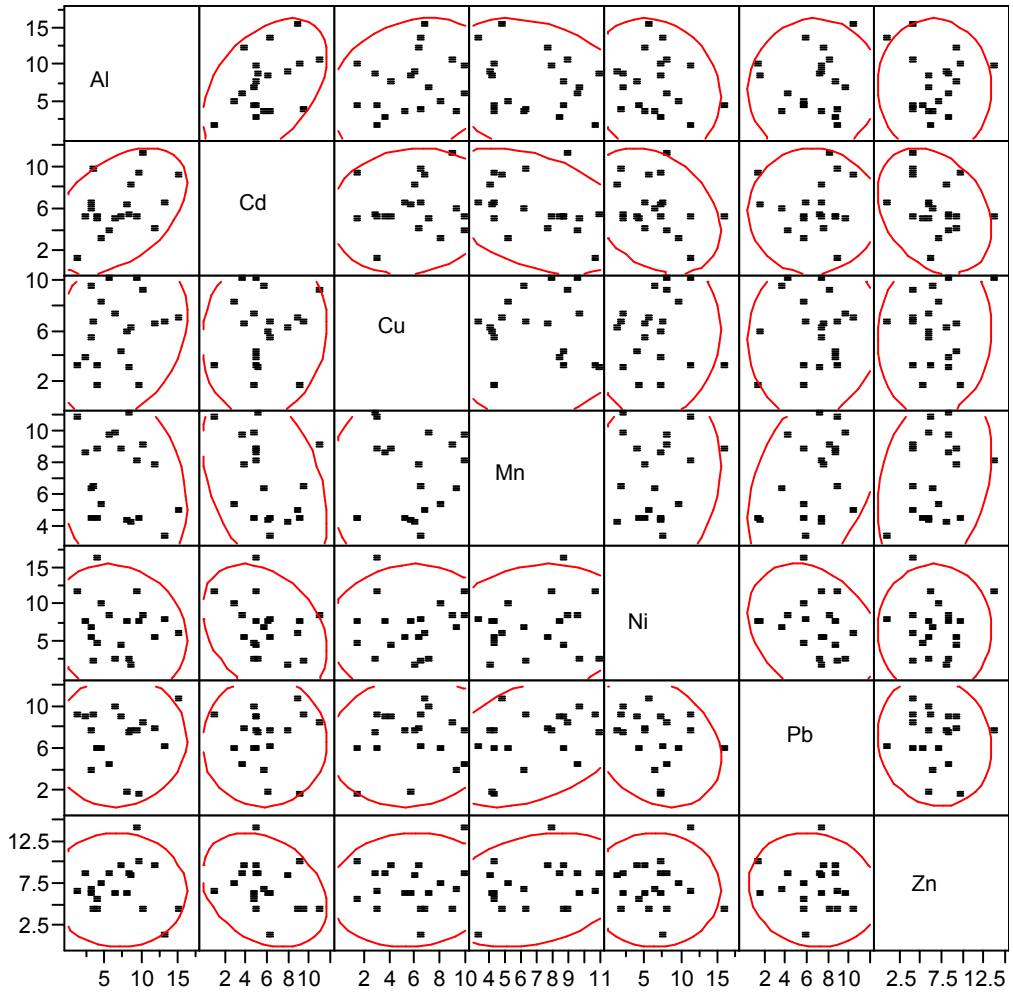
Wilk's Lambda=1,F=14 21,344df

**PPB**

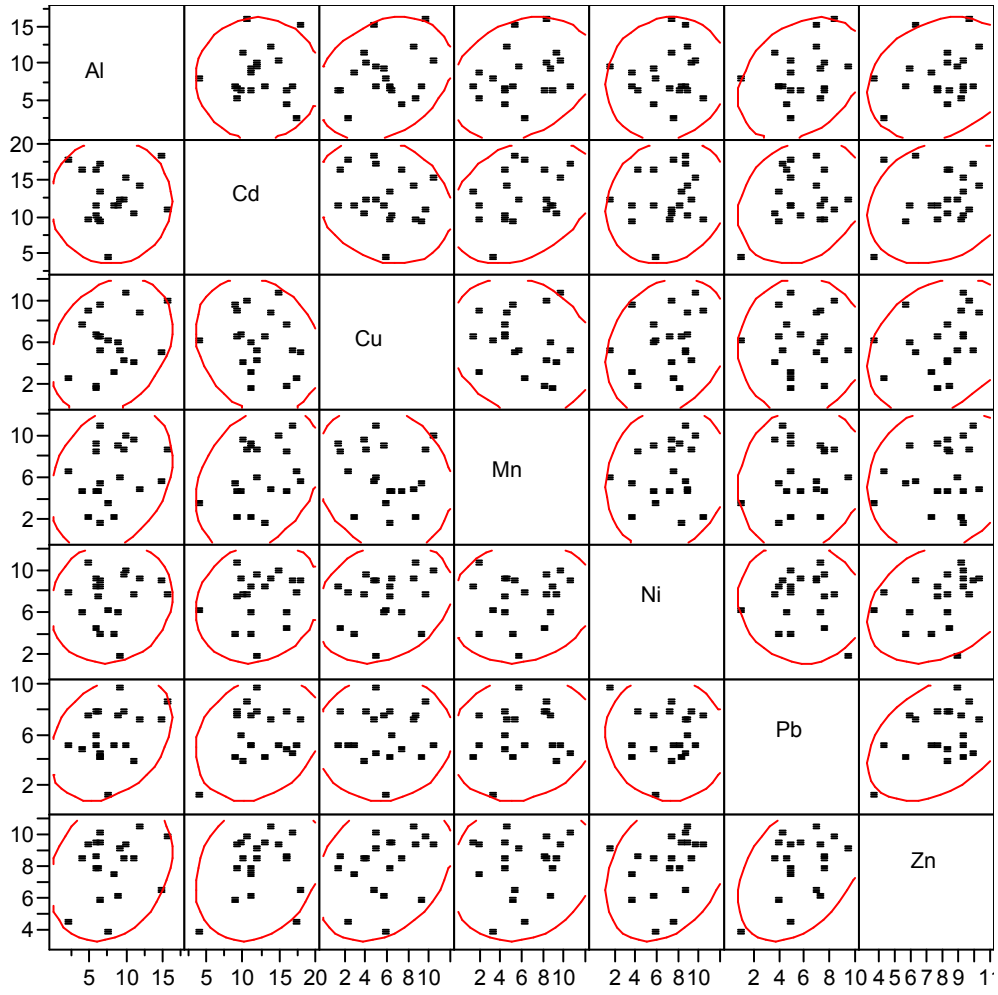



**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 64,024df



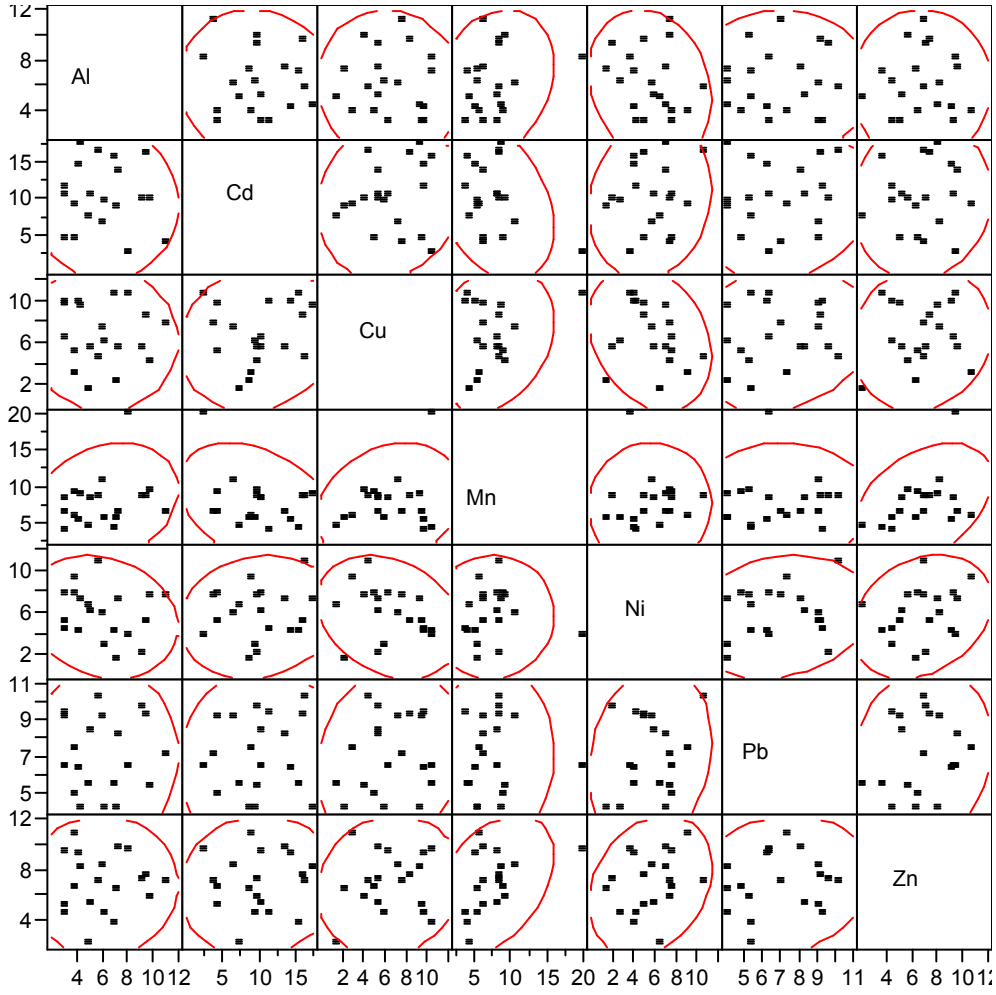

***Bacillus subtilus***
**Capsule**
**95% confidence ellipses of the multivariate mean**
Wilk's Lambda=1,F=14 25,461df



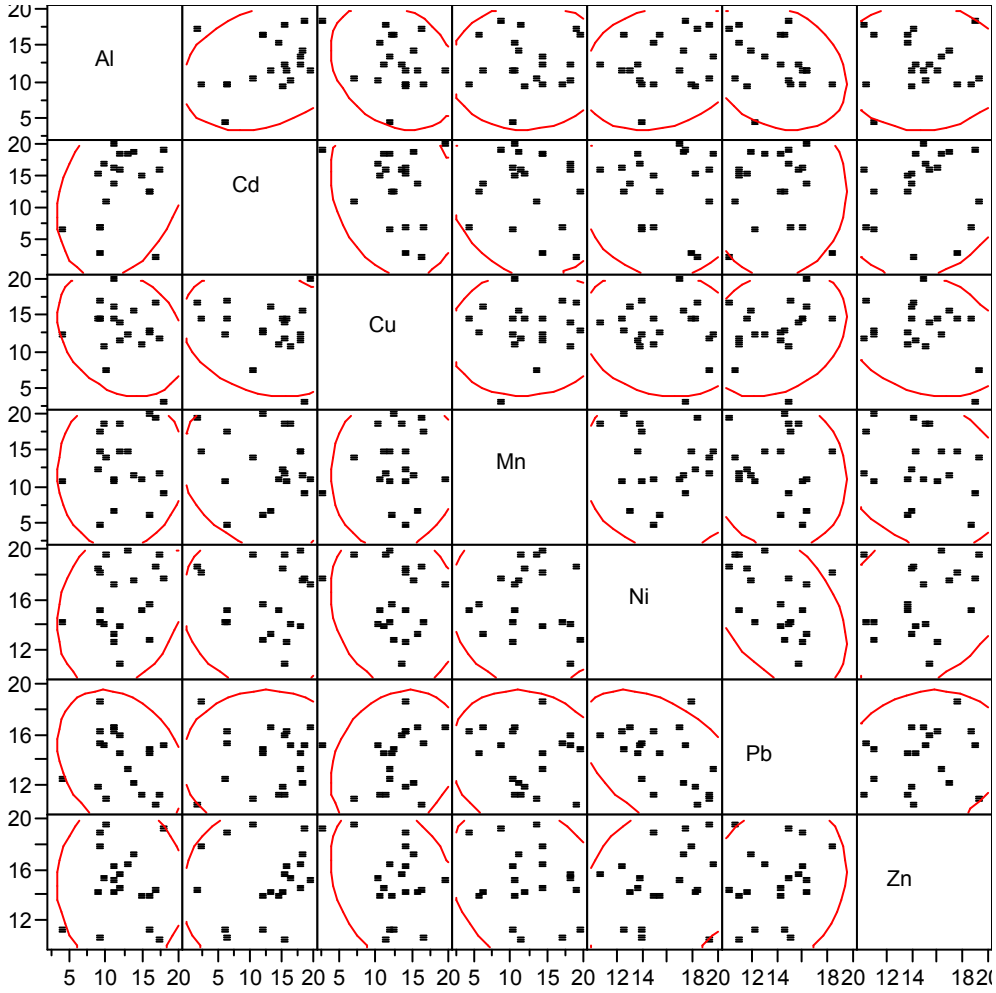

**Cell Wall**

**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14  5,635df




**Cytoplasm**
**95% confidence ellipses of the multivariate mean**
Wilk's Lambda=1,F=14  19,377df

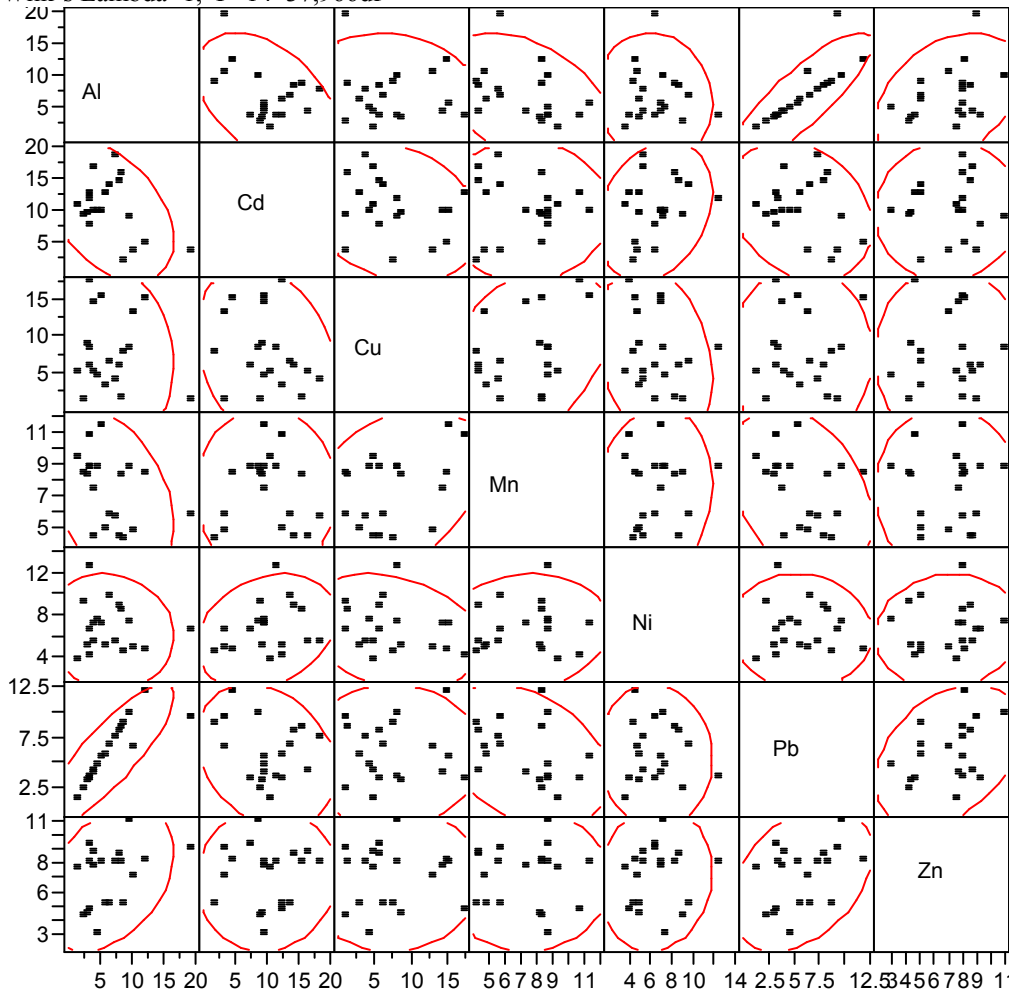



**PPB**

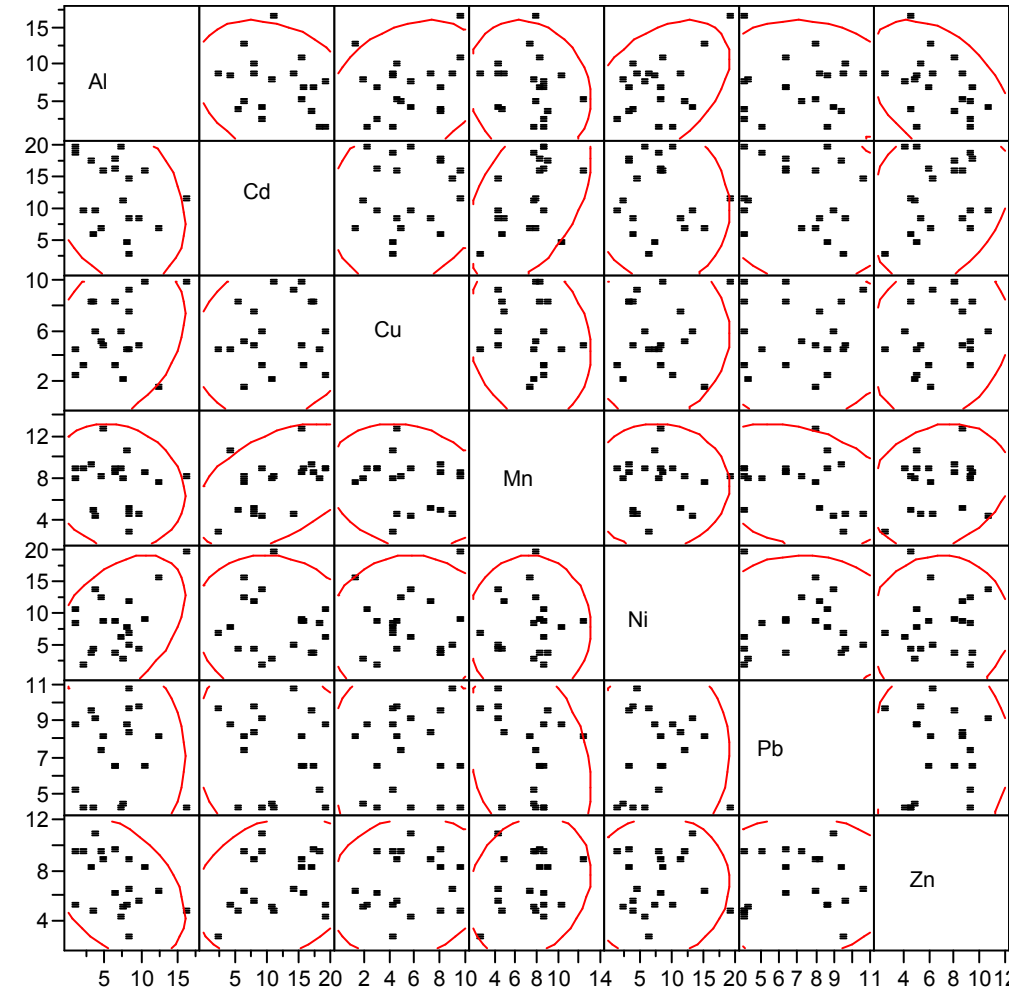
**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 12,7511df

***Staphylococcus leopoliensis***
**Capsule**
**95% confidence ellipses of the multivariate mean**
Wilk's Lambda=1, F=14 37,966df



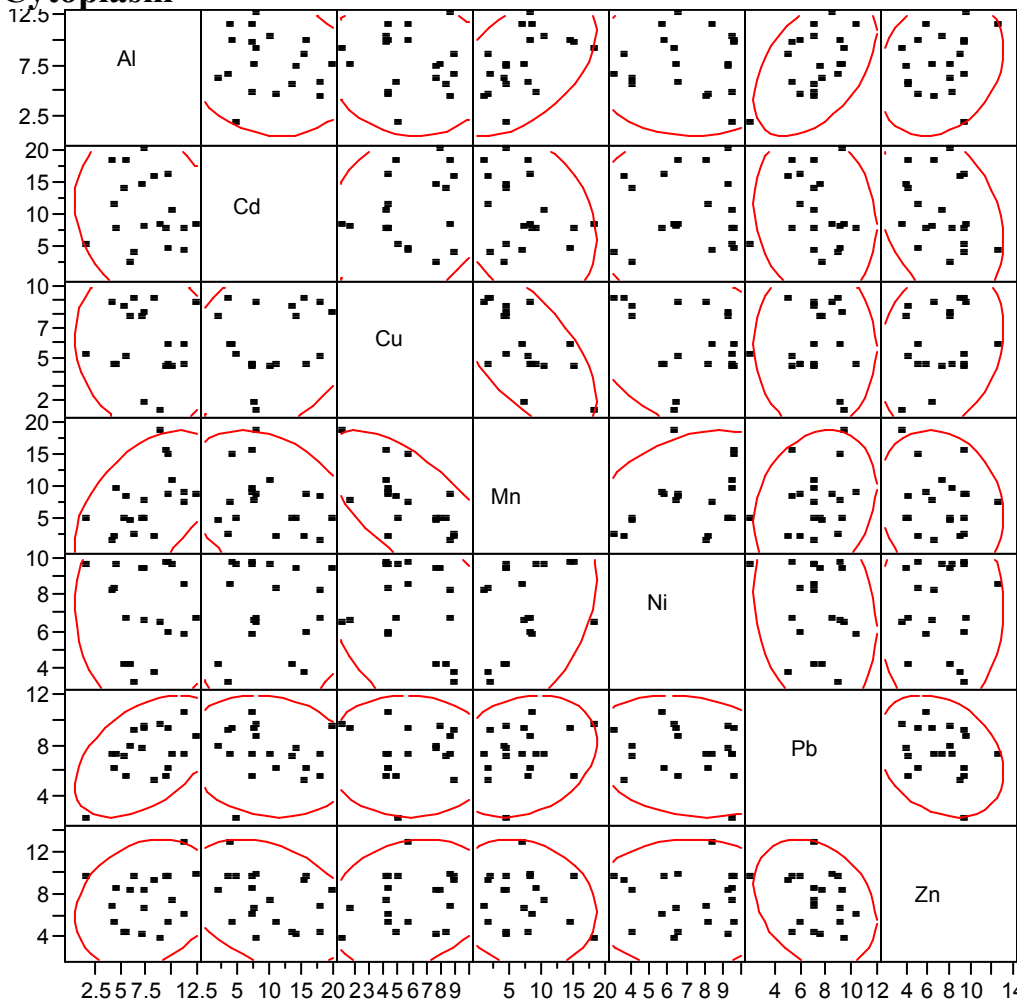
## Cell Wall




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14  19,027df

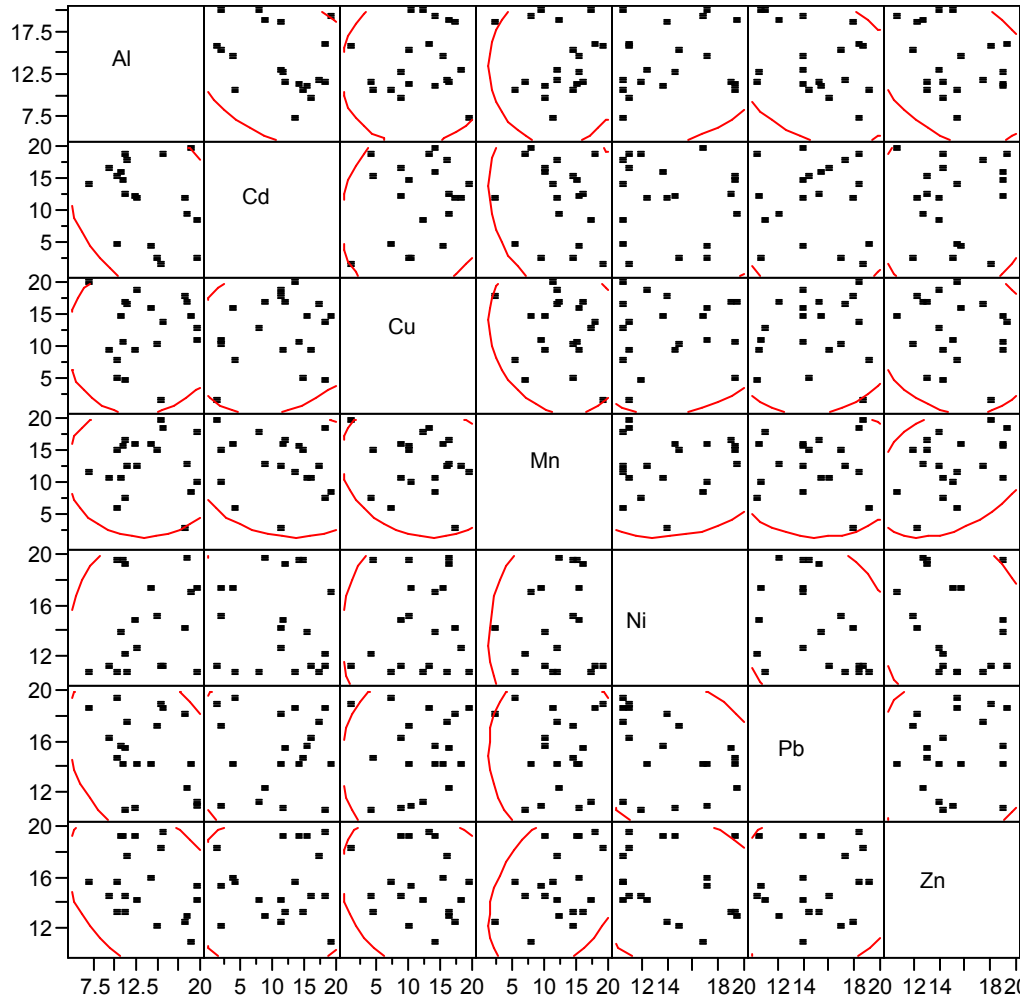
**Cytoplasm**




**95% confidence ellipses of the multivariate mean**

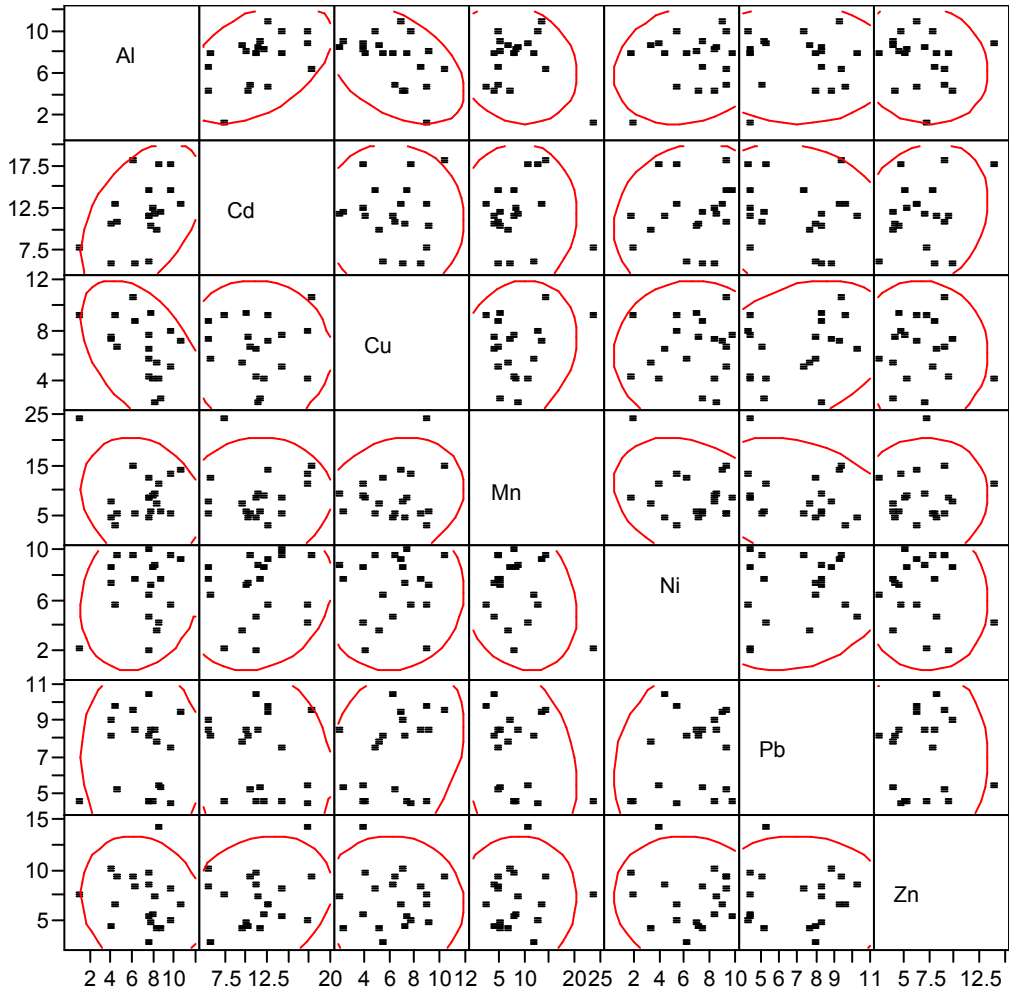
Wilk's Lambda=1,F=14 12,983df

**PPB**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 13,485df



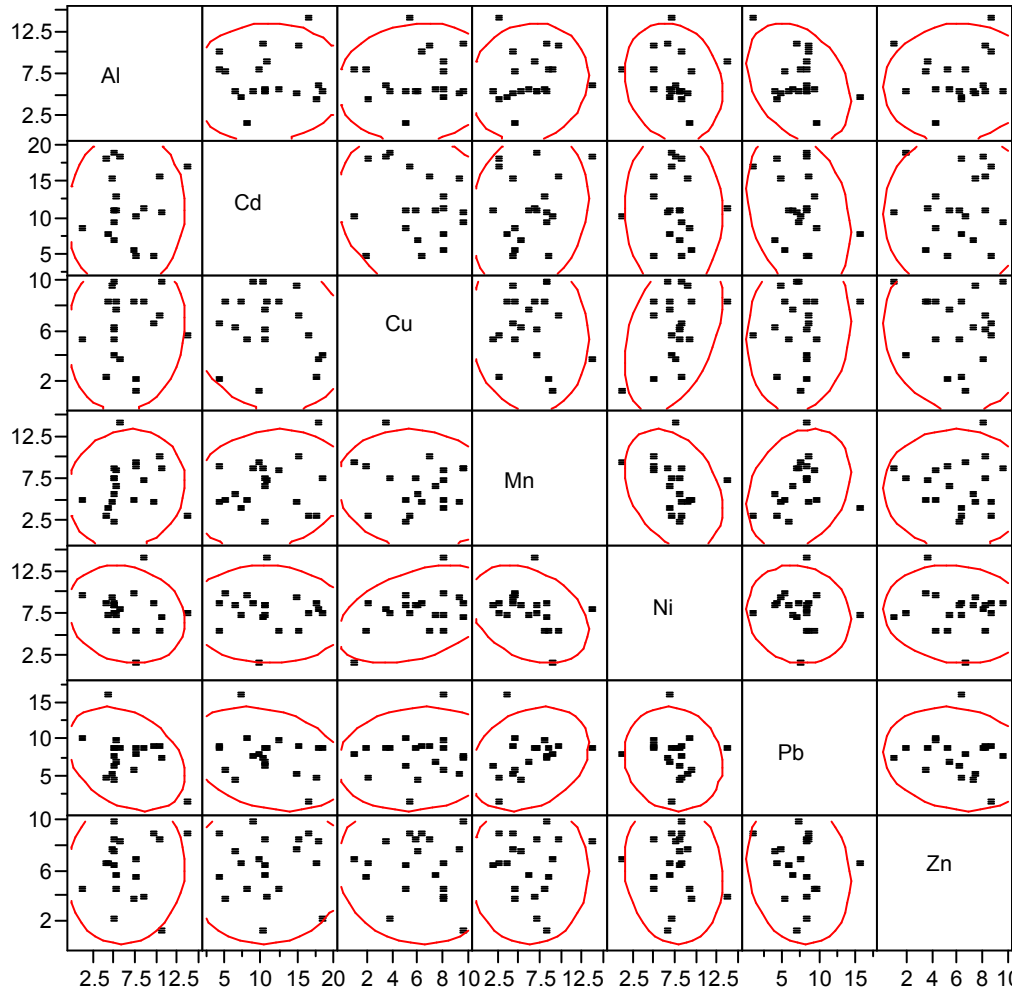

***Acinetobacter calcoaceticus***

**Capsule**

**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 12910df

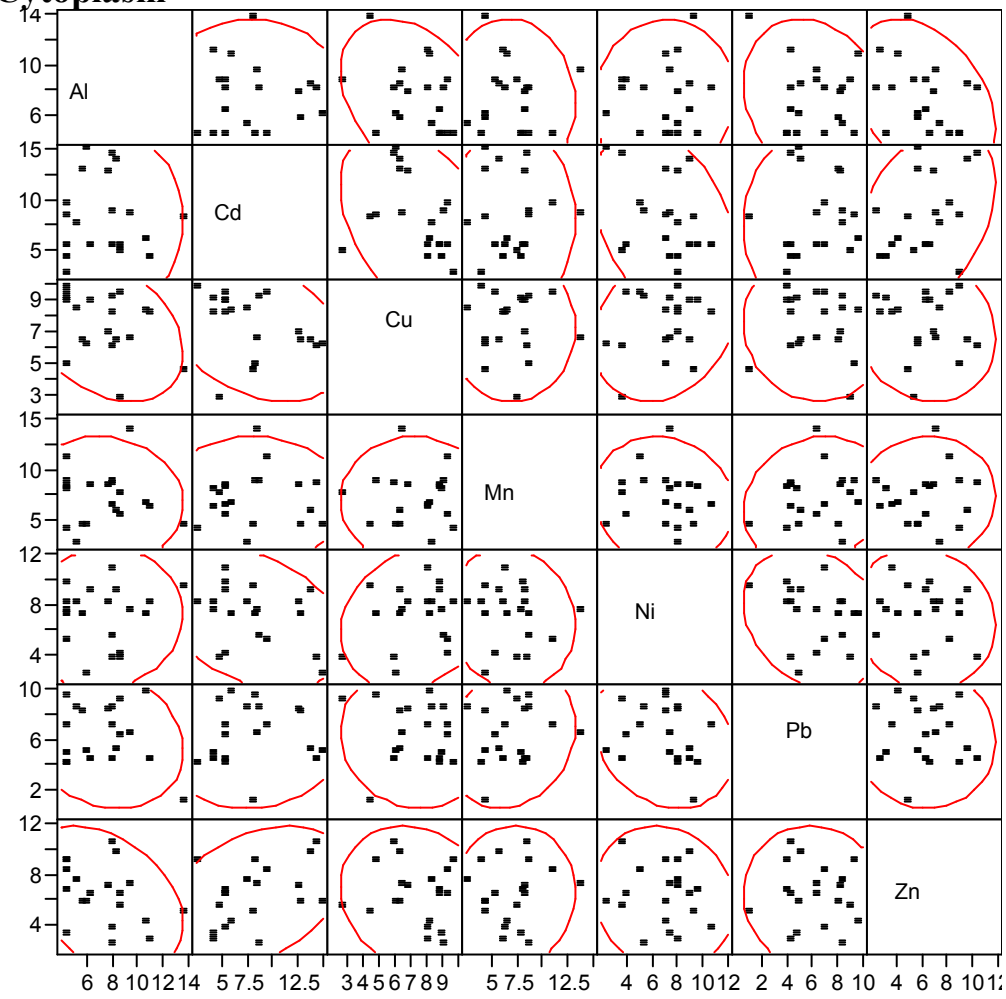
## Cell Wall




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 5,951df

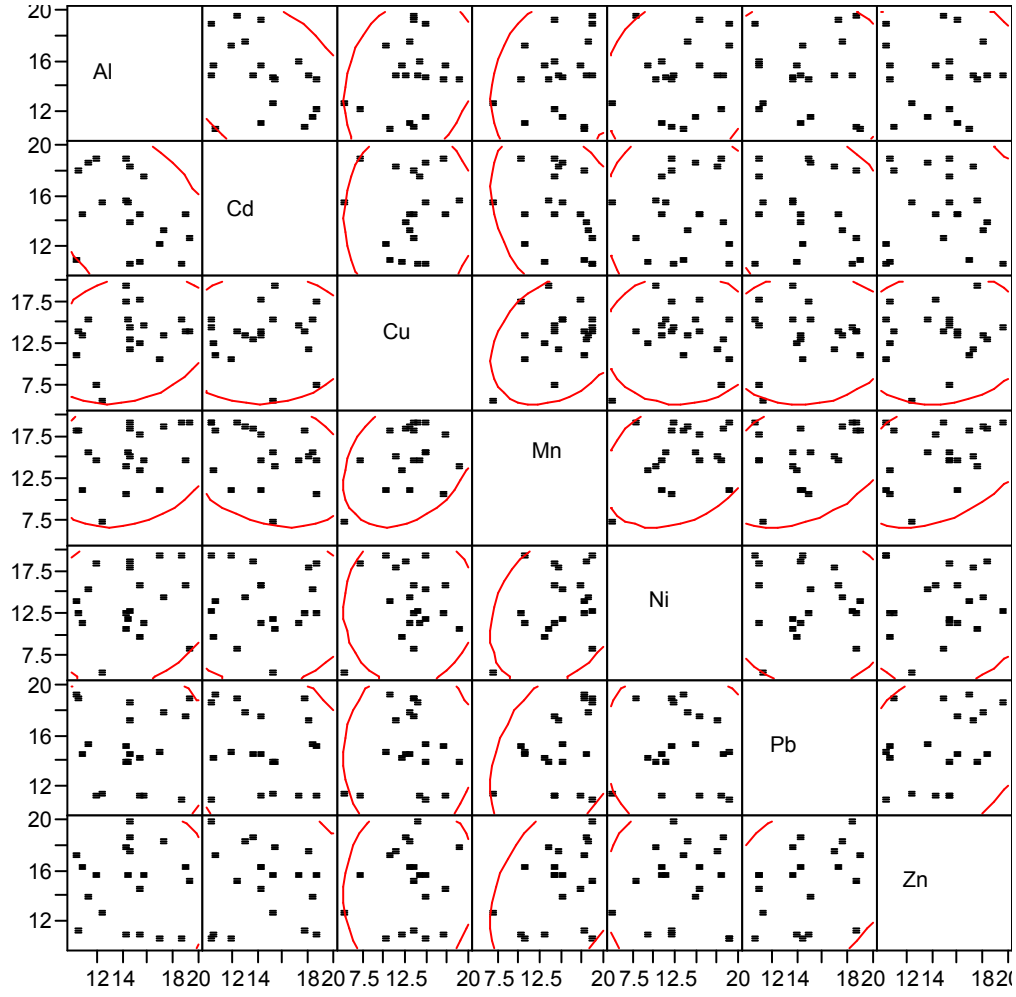
**Cytoplasm**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 16,238df

**PPB**



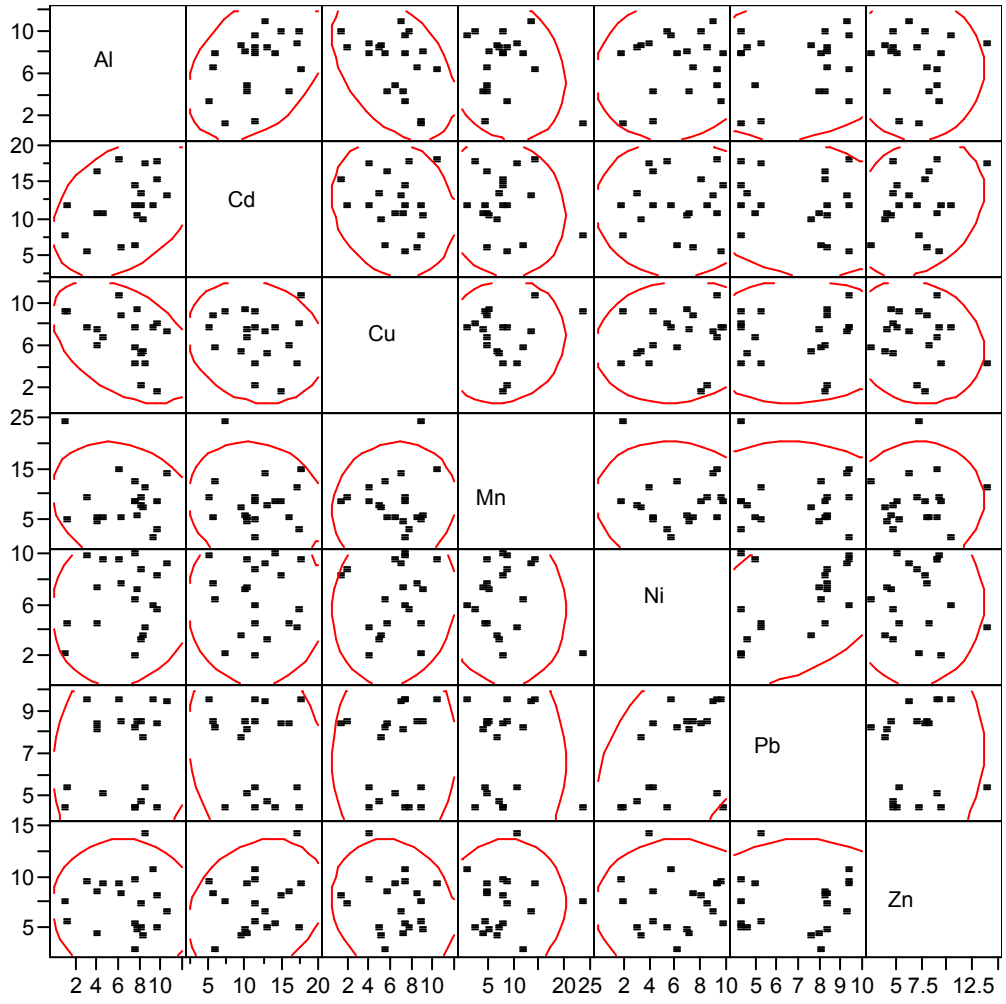

**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 35,758df

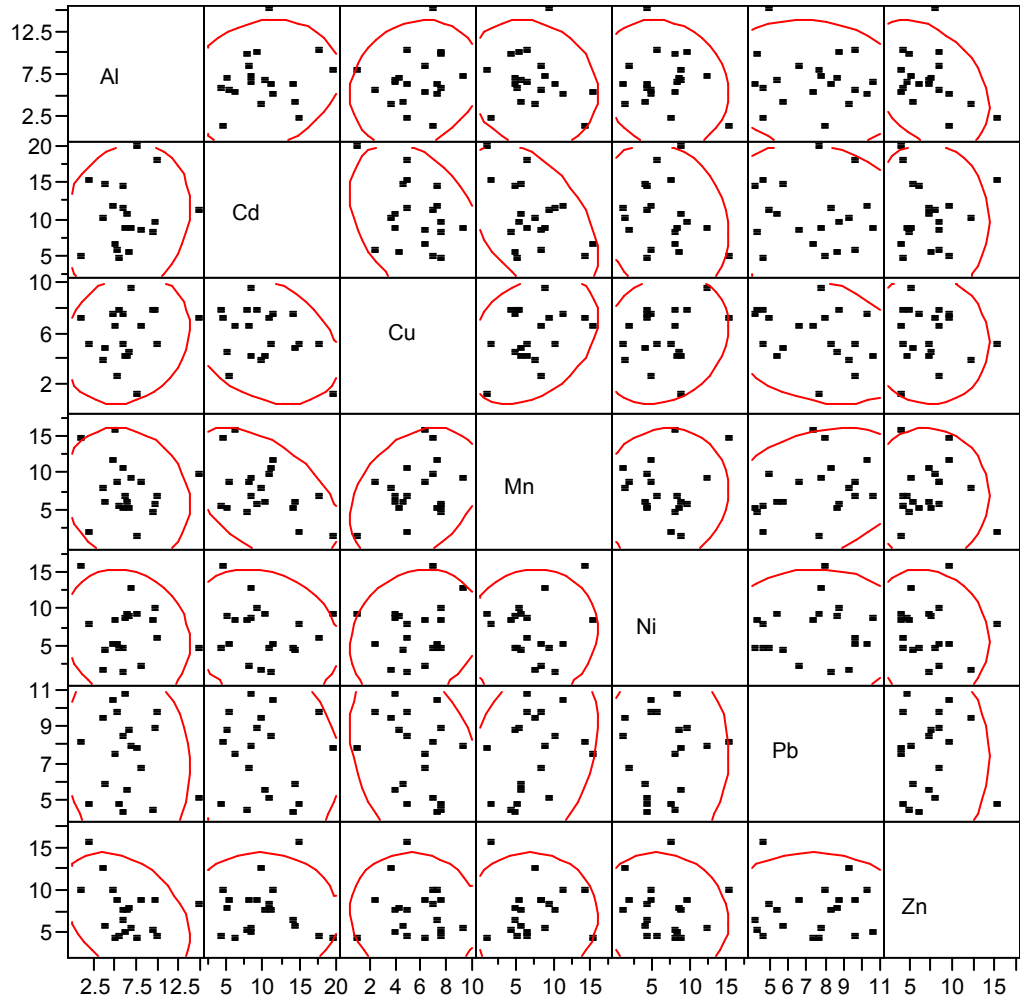
***Pseudomonas aeruginosa***

**Capsule 95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14  28,420df



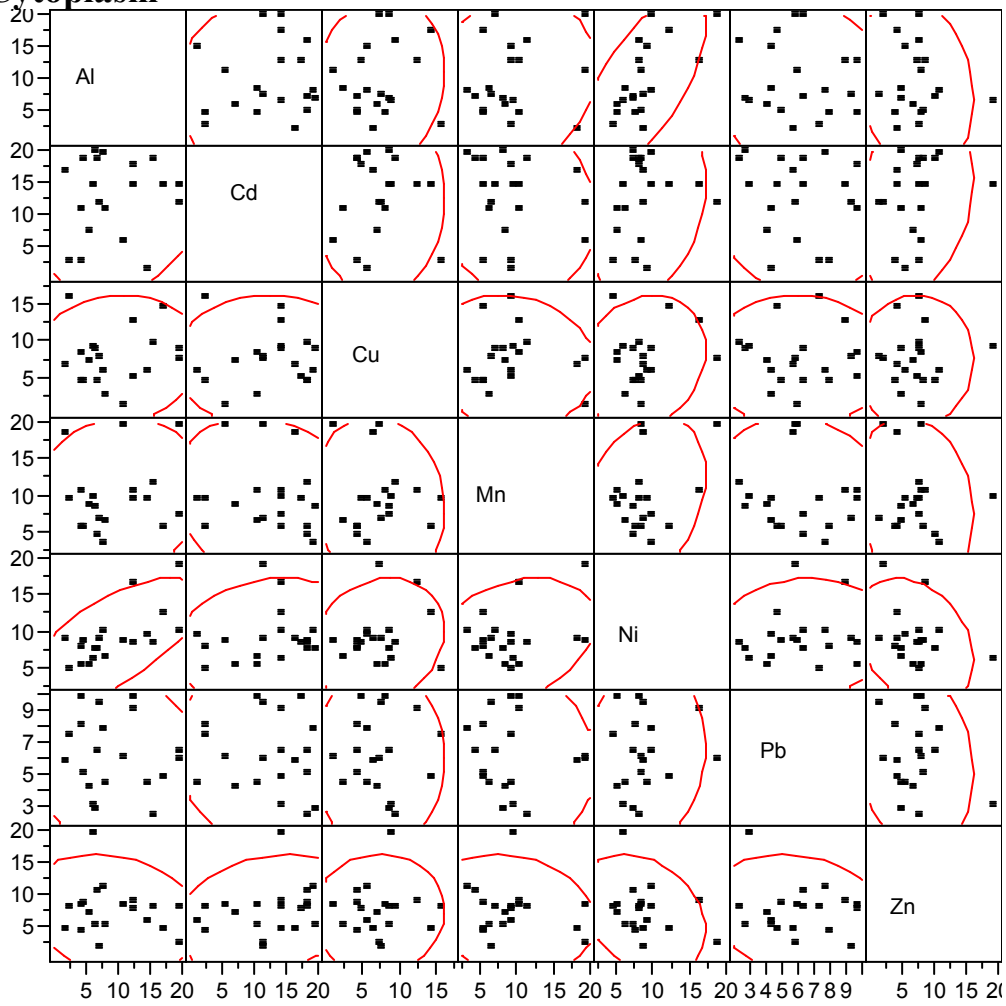
## Cell Wall




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 11,905df

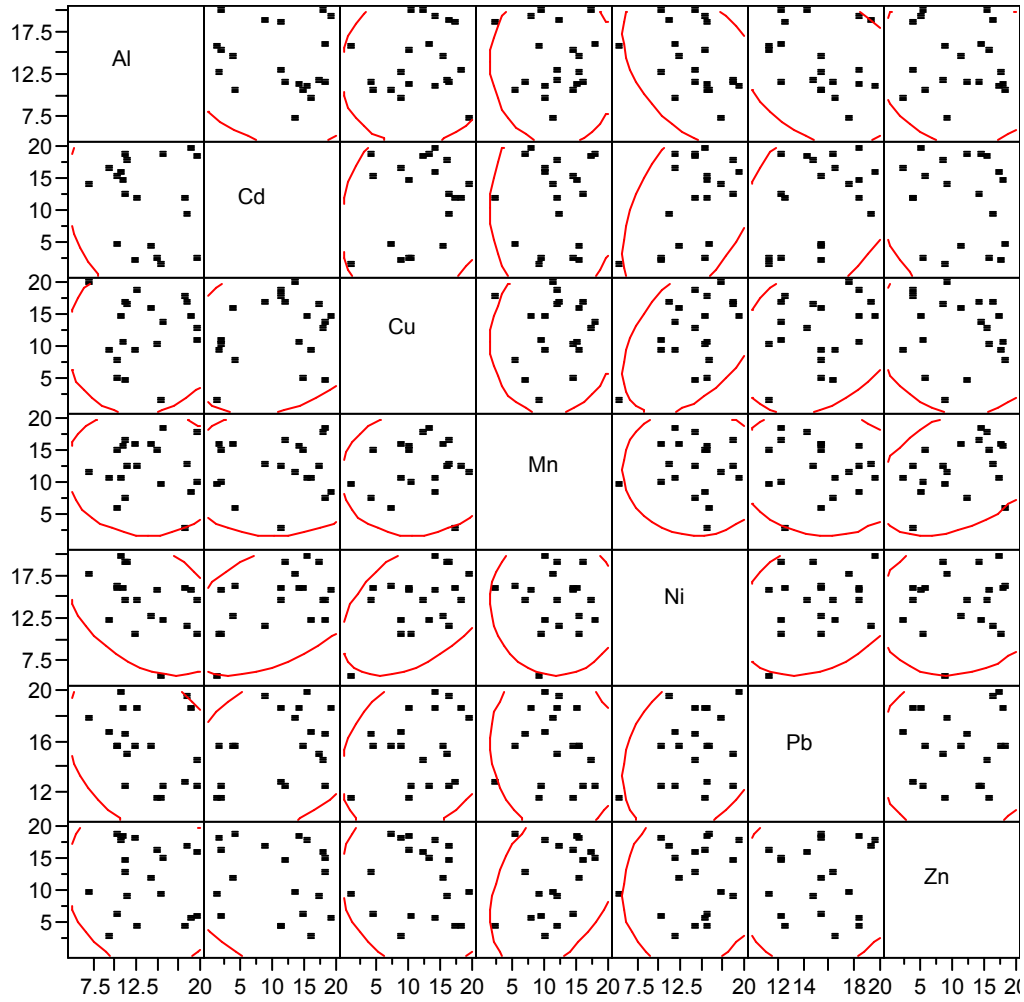
**Cytoplasm**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 13,314df

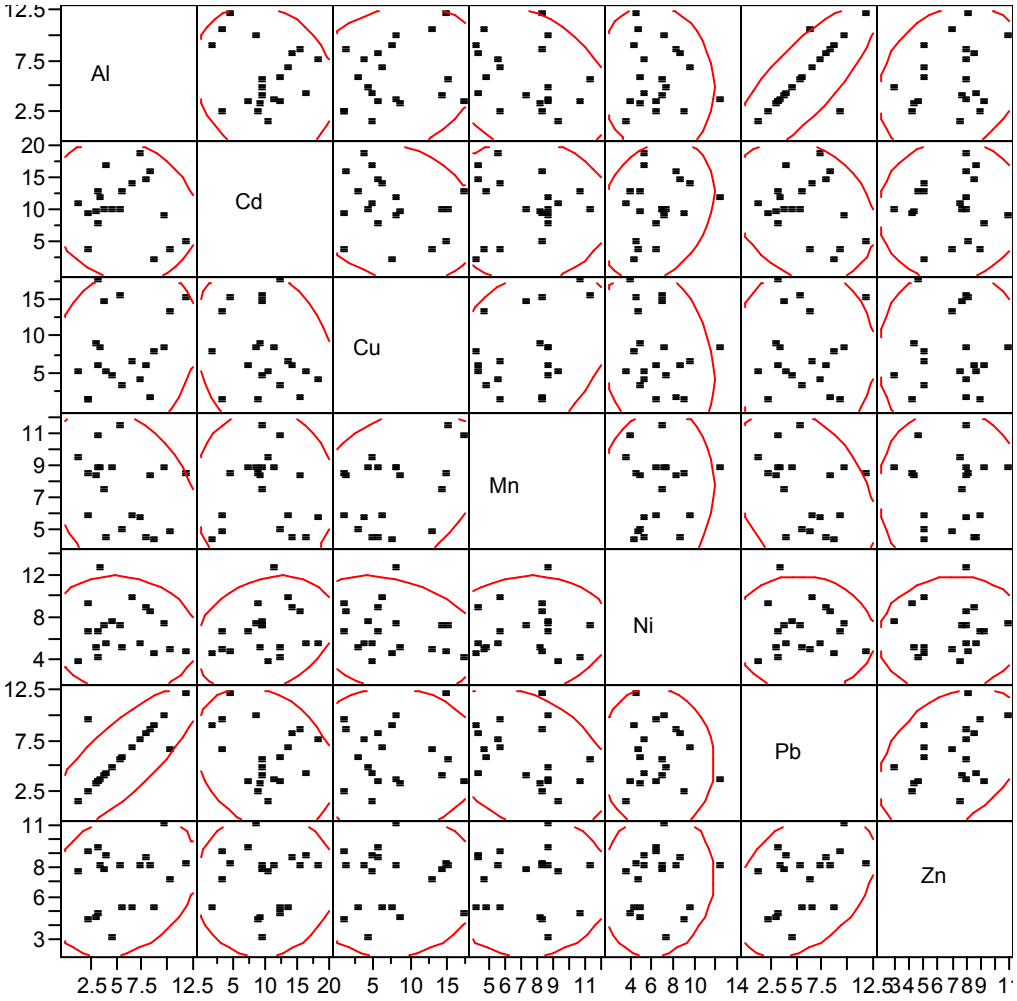
**PPB**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 2,187df

***Saccharomyces cerevisiae***
**Capsule**
**95% confidence ellipses of the multivariate mean**
Wilk's Lambda=1,F=14  9,145df



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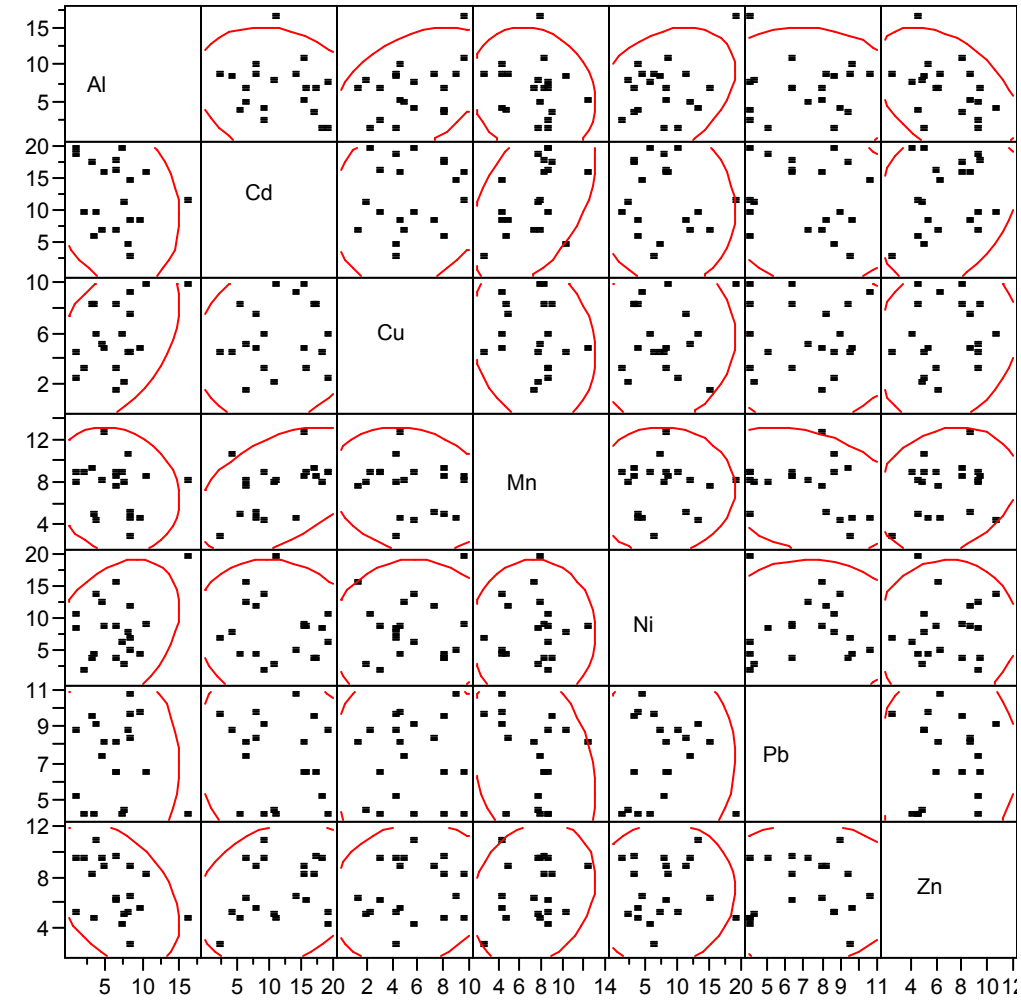
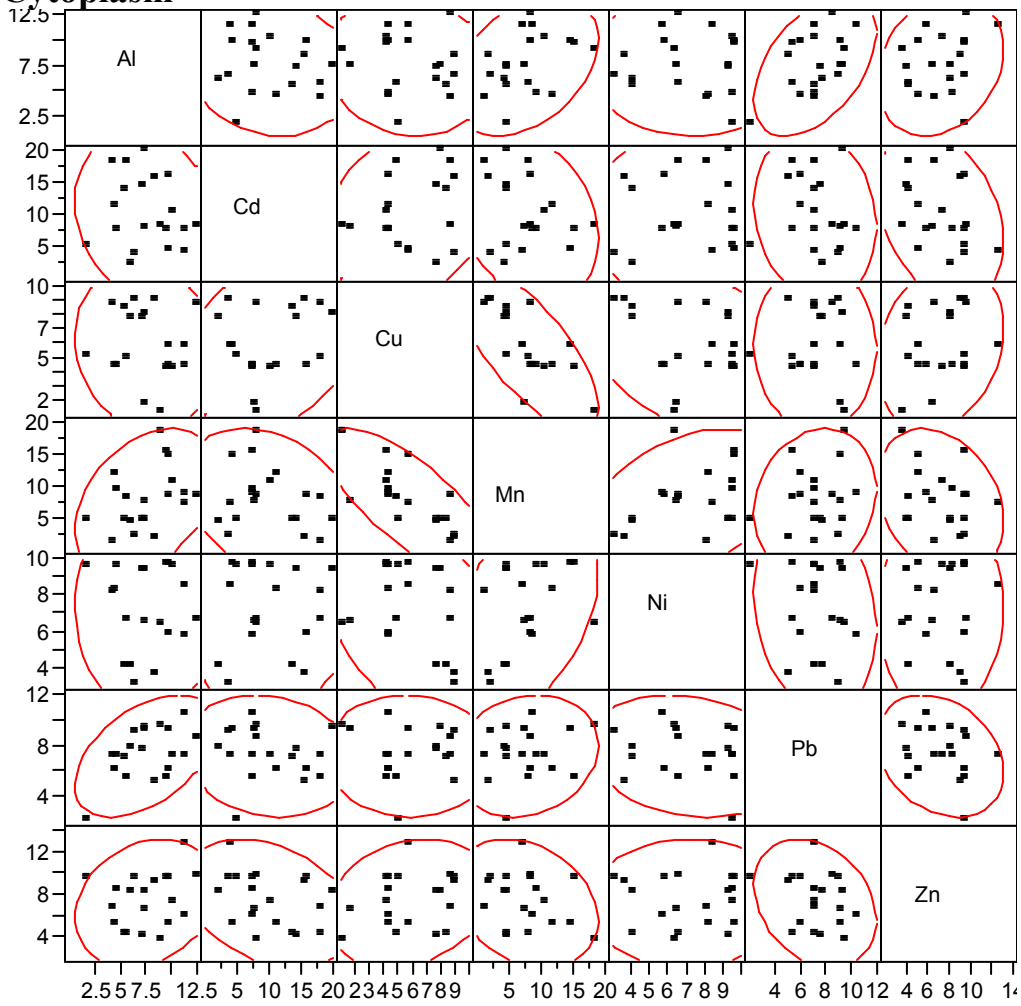



**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 1,919df

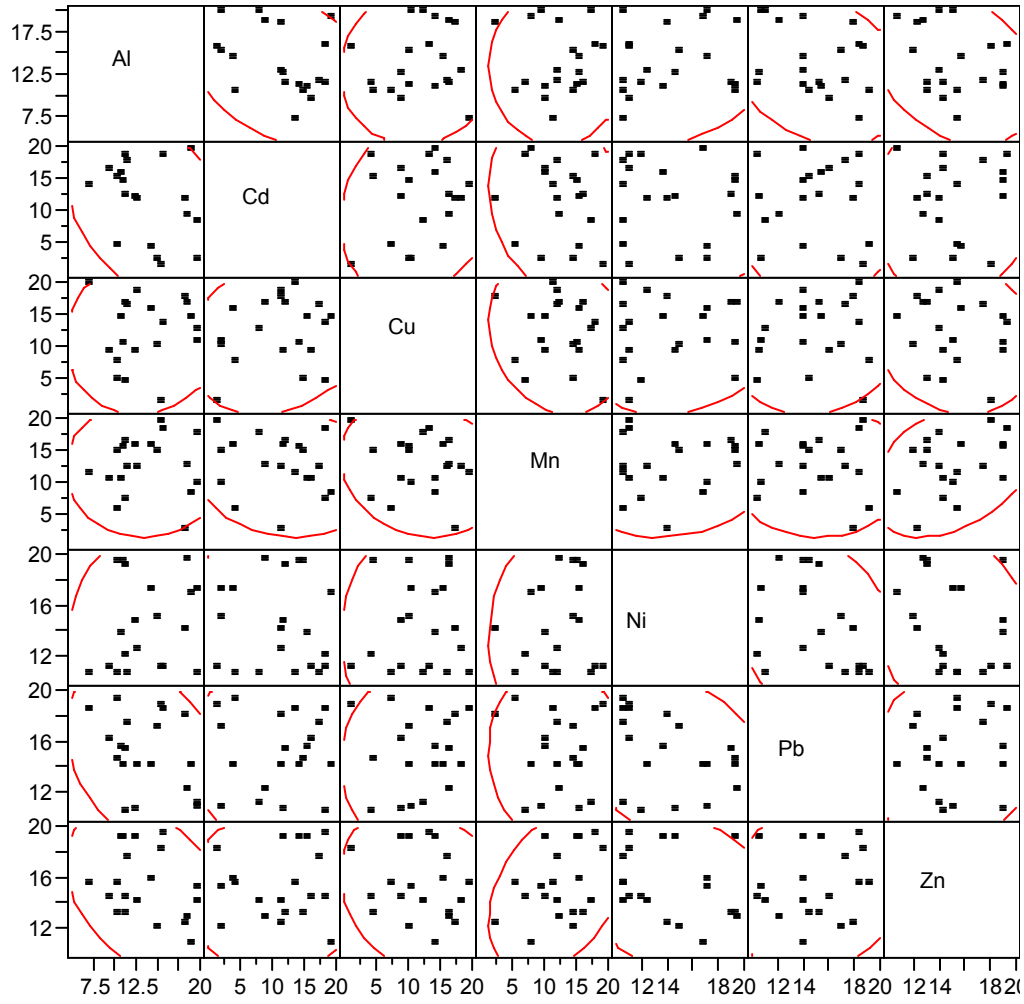
**Cytoplasm**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14  1,210df

**PPB**

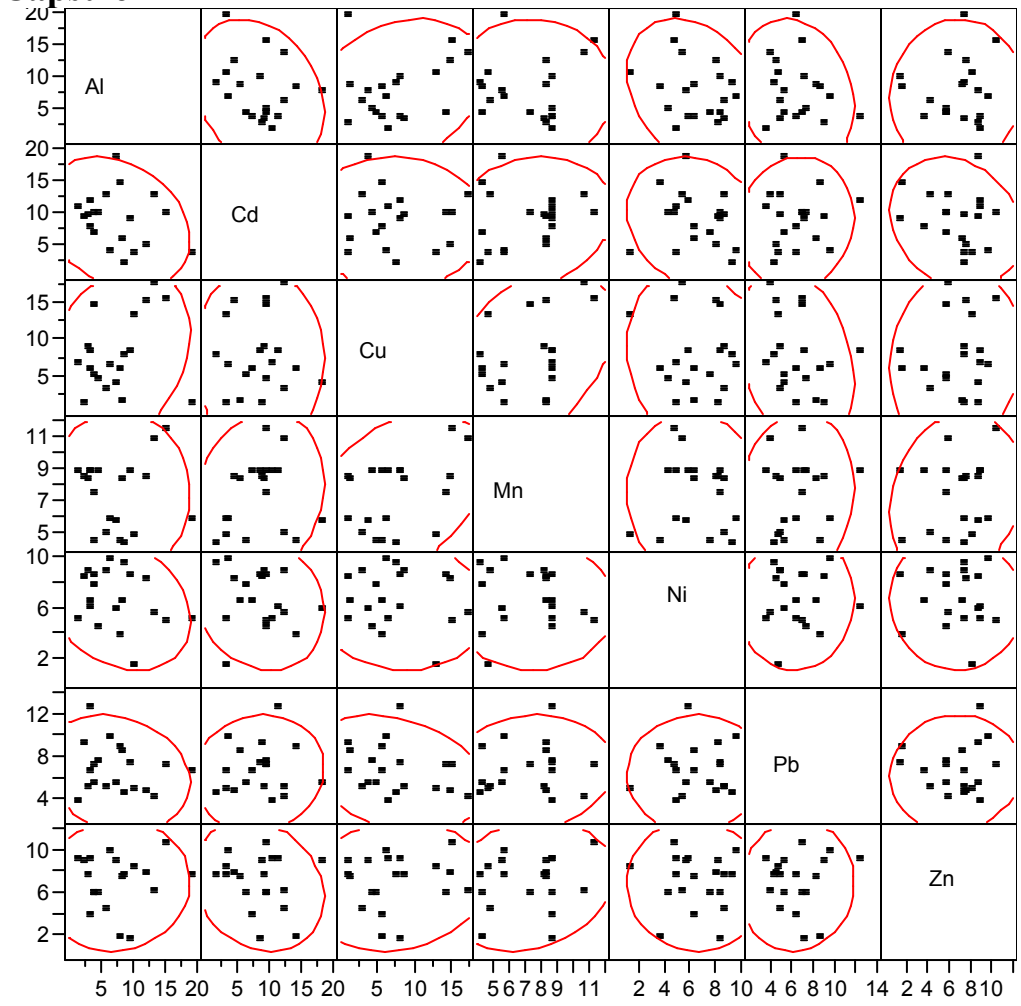



**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 5,183df

***Rhodotorula rubra***

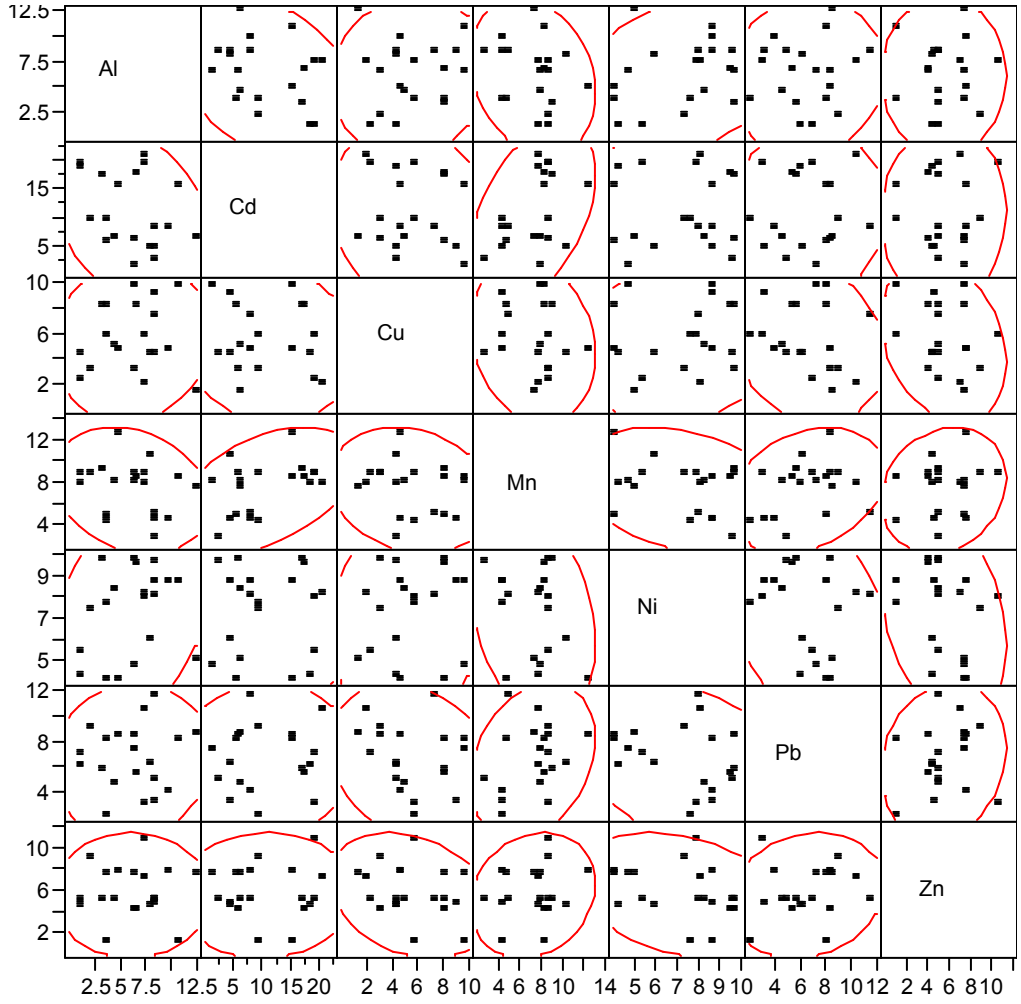
**Capsule**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 2,436df

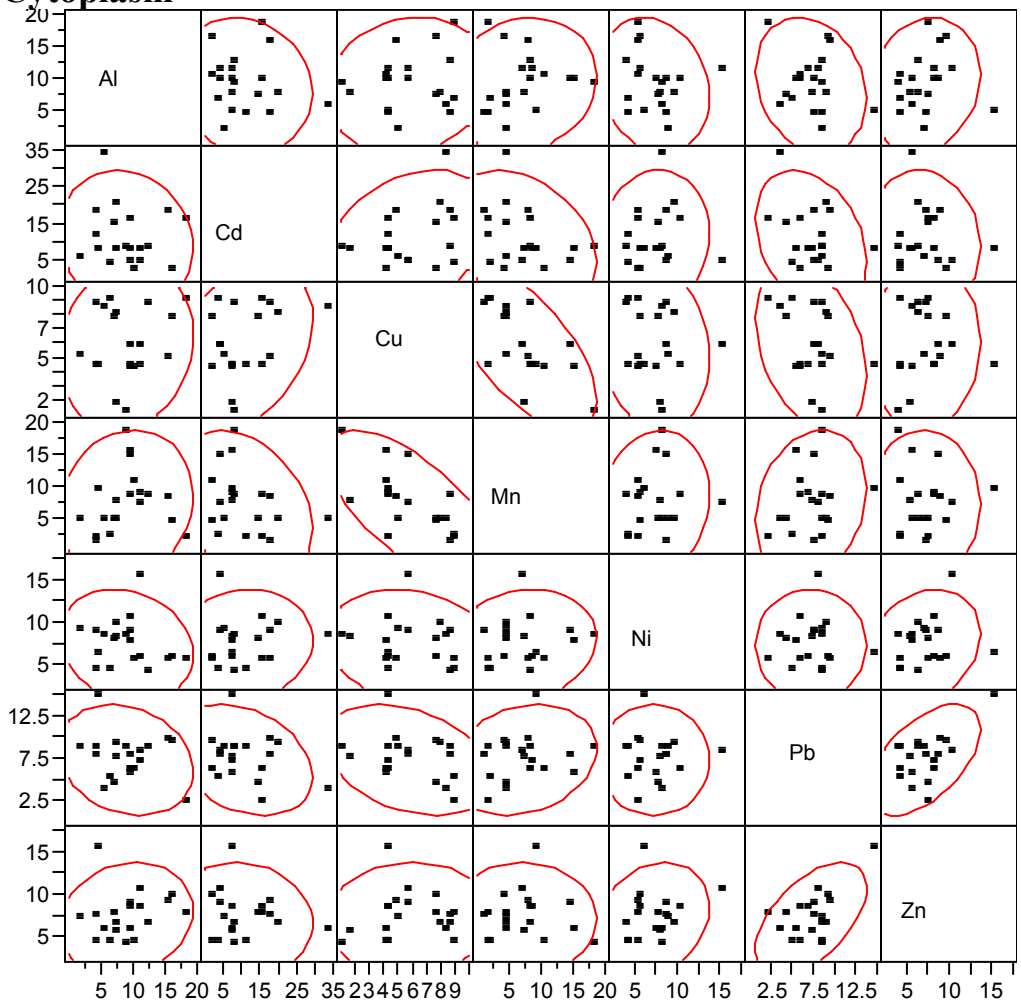
**Cell wall**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 9,209df

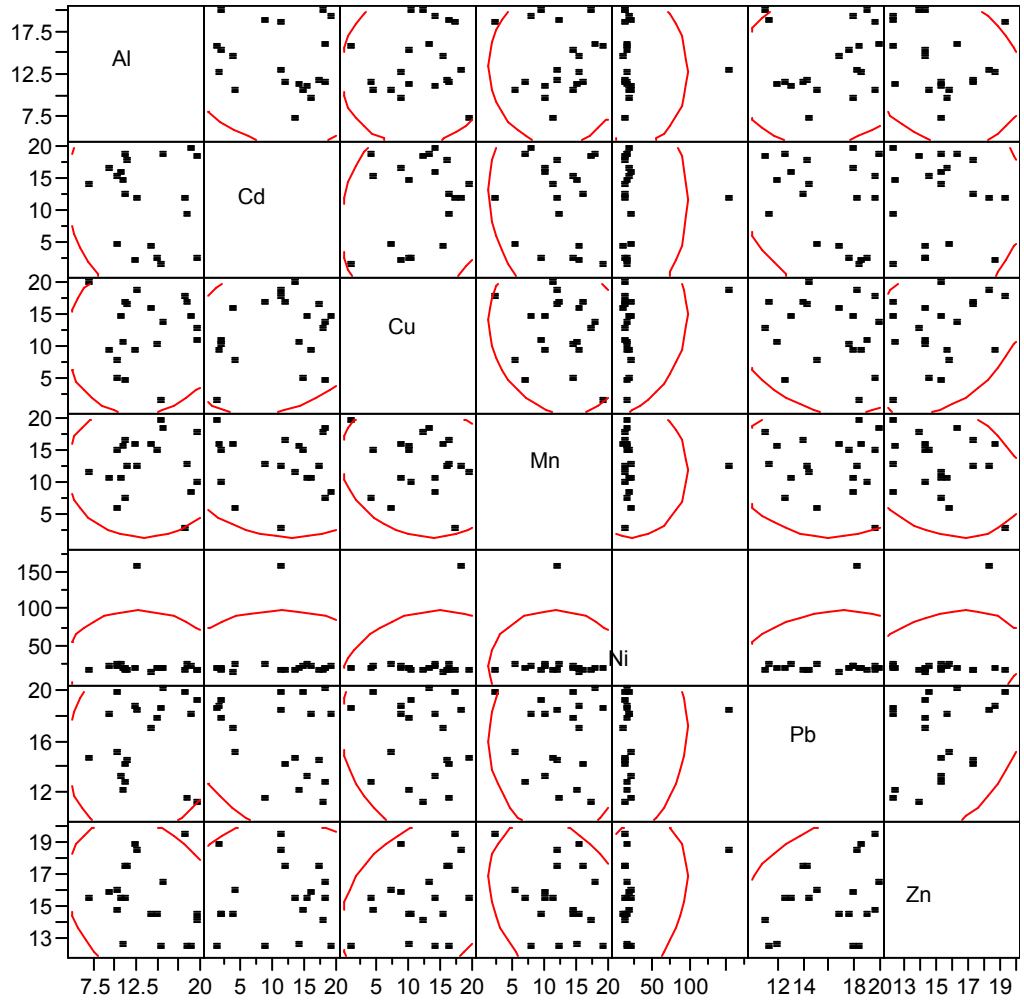
**Cytoplasm**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 15,402df

**PPB**

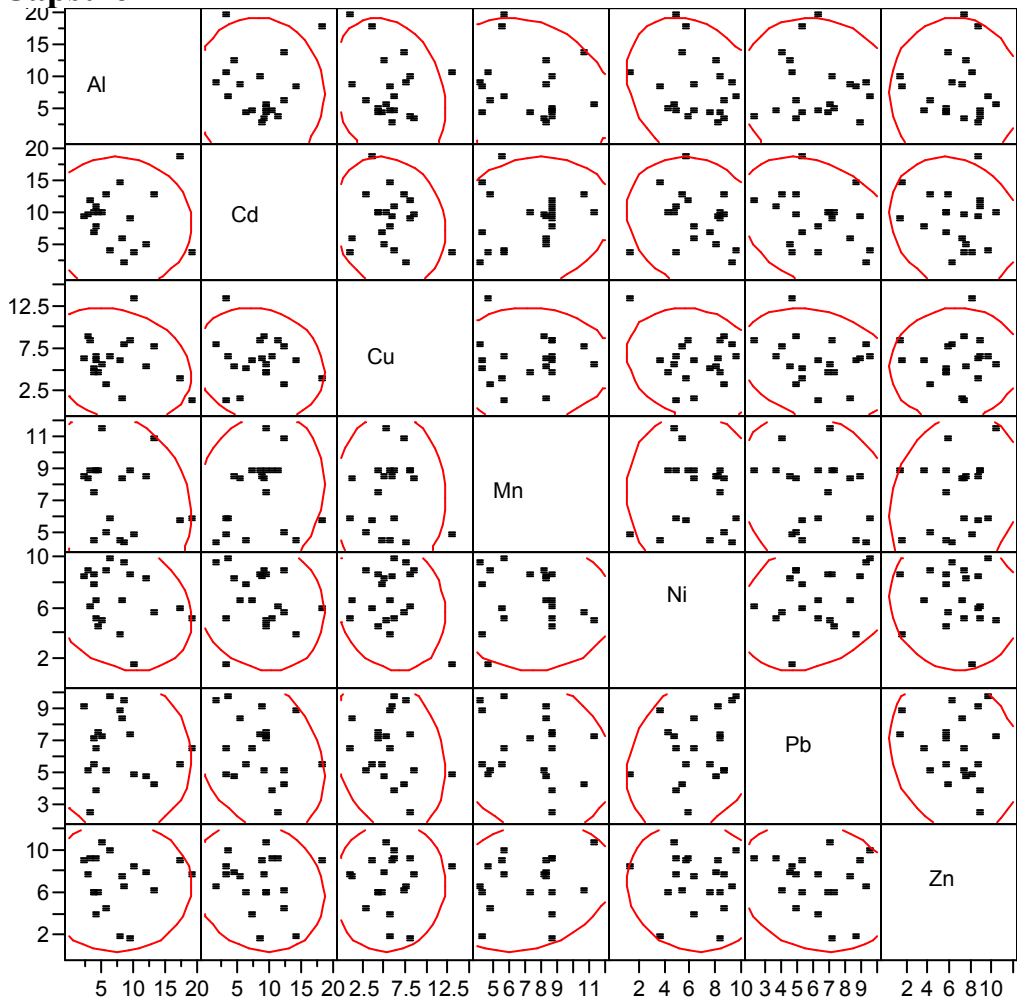



**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 10,972df

***Escherichia coli***

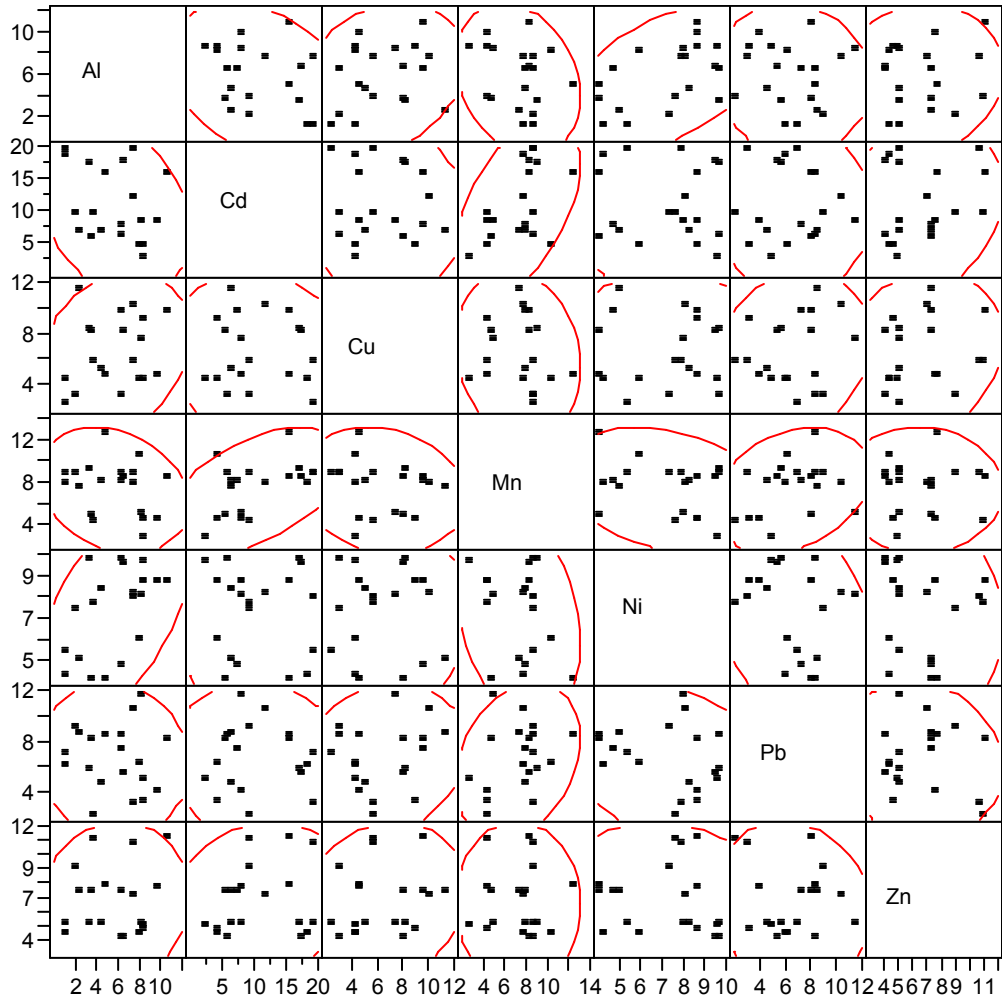
**Capsule**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 3,799df

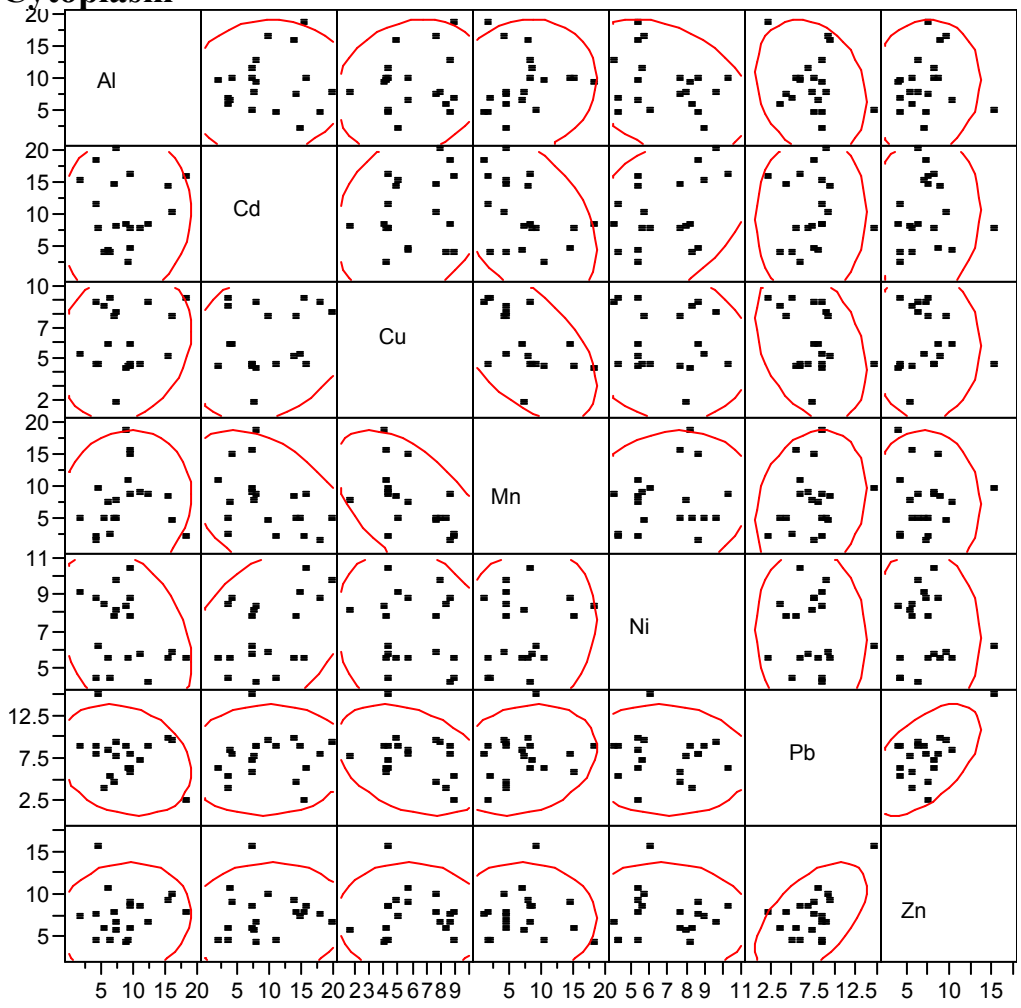
## Cell Wall




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 7,081df

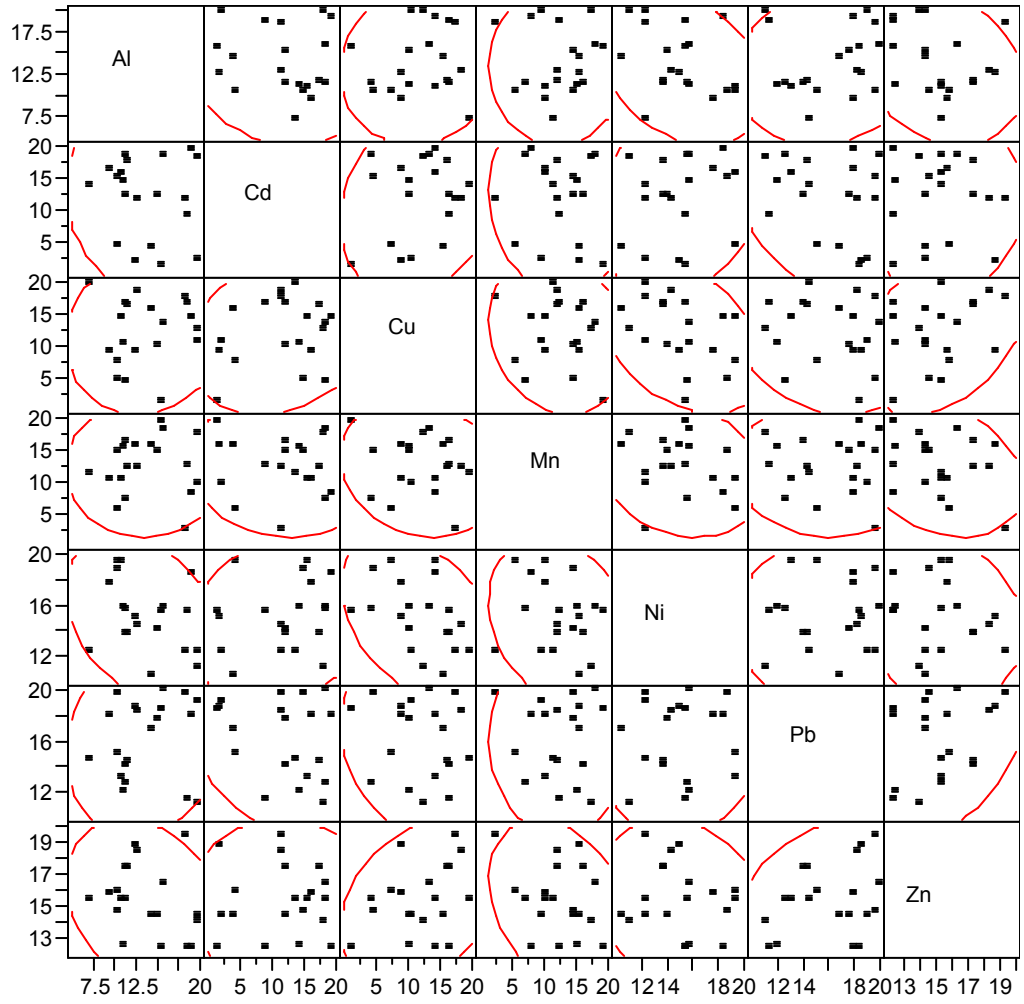
**Cytoplasm**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 9,322df

**PPB**




**95% confidence ellipses of the multivariate mean**

Wilk's Lambda=1,F=14 10,043df

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