NON-EARTH-CENTRIC LIFE DETECTION

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We search for life routinely on our own planet, thinking it unremarkable that we easily recognize it when we see it. And though every high school biology course seems to begin with the question "what is life?" we have struggled to achieve a definition for life that is universally accepted and based on measurable terms. So, in fact, it actually is remarkable that, while we know it when we see it, we have yet to reach consensus on what exactly life is. We have explored the most extreme of environments, extending what we thought to be the limits of life's hardiness and tenacity. We have learned to interrogate the human body, learning more and more with less and less invasive techniques, diagnosing disease with remarkable speed and accuracy. We have developed tools with exquisite sensitivity and specificity to identify organisms and their remains, often detecting evidence for life at spatial scales as small as a single bacterium.

But what if we were looking for life elsewhere, without the assurance that it was Earth-like, and without the aid of the Earth-centric clues that we have come to rely upon? This is the challenge that we have presented to ourselves in the Center for Life Detection at the Jet Propulsion Laboratory. Can we shed our Earth-centric preconceptions to assure that, should we encounter life outside our own planet, that we not miss it? The task is made difficult because of our training in the sense that we burden ourselves with the assumption that we know what life is and how we might detect it. However, it should be possible to glean some general features of life through the lessons learned from the study of Earthly environments. Our hope is that life will, bit by bit, reveal the clues that will allow us to piece together enough evidence to recognize it whenever and however it presents itself. Indisputable evidence is measurable, statistically meaningful and independent of the nature of the life it defines. That the evidence for life be measurable is a fundamental requirement of the scientific method, as is the requirement for statistical significance, and this quantitation is what enables us to differentiate the measurable criteria of candidate biosignatures from a background (host environment). Defining the evidentiary criteria in this way obviates the Earth-centrism inherent in limiting the definition of a biosignature to the chemistry, structures and behavior of known life. DNA, proteins and other recognizable biochemicals; bits of carapace, shell, bone, etc., by which we recognize many Earthly fossils in the rock record would not be required to recognize life. And eliminating Earth-centric bias from life detection strategies increases the probability that we will not only know it when we see it, but be able to prove we see it, as well.

Developing such a program has other potential advantages. Because the detection methods will ultimately be automated, rapid, and unbiased, they may, in fact, be useful for the detection of life on our own planet. Once the approach is defined, it can be tested on Earthly life and fossils, affirming its utility through ground truth analysis with proven methods. Once we develop robotic instrument packages based upon these methods, the instruments can be deployed beyond the Earth for the identification of life, even if the alien life is unfamiliar to us. Finally, when samples are returned from Mars or other bodies in the solar system, non-Earth-centric methods for life detection will certainly be necessary for the analysis of recovered samples.

APPROACH AND STRATEGY

The approach for detecting life on other planets must prove viable on the Earth. We employ the following protocol:

1. Define life in measurable terms
2. Characterize life's biosignatures
3. Determine the best way to measure these biosignatures
4. Develop and test the measurement instruments and methods
5. Build a biosignature database
6. Hunt for life, comparing the observations to the database
7. The result should be a decision: life, past life, non-life
8. Confirm non-Earth-centric methodology, that is, ground-truth the non-Earth-centric methods with standard methods of life detection

This approach (also shown in Figure 1) allows one to define the strategy for life detection that accomplishes a number of things, and is compatible with constant revisions and redefinition. We are continuously refining the notion of what is the best way to measure biosignatures as we develop new technologies and methods to do so. Better detection limits can enable us to consider biosignatures we didn't know existed prior to technological evolution, so these advances extend the breadth of the database. Moreover, the expansion of both the repertoire of life detection methods and the resultant database, in turn, enables a better statistical basis from which to employ a neural network for making the classification decision of step seven. The detection of life is an evolutionary process, a model that should be flexible enough to allow for adaptation to new conditions as knowledge increases, sometimes altering initial assumptions. The strategy we have chosen is one that stresses the ability to define the measurables, then using physical, chemical, and statistical methods in an integrated way to define life in its most basic form. Ultimately one can make an informed decision on the basis of fundamental measurements. Our strategy also assumes that no single measurement will be adequate; e.g./ a number of measurements taken together will form the basis for the decision, or in many cases, inform the scientist of other measurements that are needed.

The database of biosignatures and life will be composed of measurements gained through the study of living systems on Earth, and all new methods can be continuously tested and refined through studies of Earthly organisms, living and dead specimens, as well as fossils of past life. Such an approach ensures that field testing support lab-based assertions regarding detectable biosignatures. If we can look for life in the field as successfully as we can in the laboratory, then we can make choices regarding the deployment of in situ or sample return projects free from the constraints of technology limitations. This is important in a space-mission-oriented environment, where other factors may ultimately determine selection of one mission type as opposed to the other. Distinctions between "in situ," "remote sensing," and "sample return" can be blurred at many levels, and proximity of the analytical technique to the sample and the scientist is what often drives the distinction. If the purpose of the life detection technique is to make a statistically meaningful decision, life, past-life, or non-life, than the operative scientific criterion is the statistically meaningful aspect, not the proximity issue.

An awareness of the spatial and temporal scales over which to search is important in the development of any life detection strategy. Biosignatures may range from the sub-nanometer to galactic in scale. At this time, we really don't know the limits over which the signals of life might extend. If, for example, the biosignature is galactic in size, we'll be able to see it from the comfort of a terrestrial observatory. On the other hand, if the largest observable biosignature is about the size of a terrestrial planet's atmosphere, we will need to put an instrument considerably closer in order to detect life. Additionally, we must understand what sampling rates to use when looking for biosignatures, i.e., how often should we look? We know, from astronomical observations, that the periodic table is consistent throughout the universe so constraints are placed upon recognizable chemistry. We also know, from studying life on Earth, that the distribution of chemical elements associated with life in some volume of interest has a temporal dependence, that is, elements will diffuse away at some rate that varies with environmental conditions. Life is often said to maintain itself in chemical disequilibrium relative to the thermodynamic stability of abiotic phases such as minerals. Hence it is necessary to define the spatial and temporal scales over which this disequilibrium is manifested. And this is why sampling rate becomes important. We can be sure from what we already know of Earthly biology that life will be observable over more than one type of scale, and probably different orders of magnitude, as well. Recognition of this manifestation of biological complexity is helpful for two reasons. First, one can use complementary techniques to see both the forest and the trees. Second, the observation of one level of organization can be used as a predictor of another level of organization, and as life is detected at each successive level (both in terms of scale and magnitude), each "hit" increases the statistical confidence of the preceding observations. If one can accept the idea that biosignatures are observable over multiple scales and magnitudes of observation, than one can address the issues of limits, that is, what are the smallest and the largest biosignatures? In other words, what is the dynamic range of a biological signal? Scientists are accustomed to working in a reductionist way; we look at the smallest bits of something in order to assemble the big picture: atoms are the smallest recognizable bits of a chemical element, and cells are the smallest recognizable bits of life. Our approach seeks various scales of organization, so we ask not only what is the smallest recognizable evidence for life, but also what are the largest recognizable levels of organization? When this answer is known, the issue of proximity can be addressed.

Defining Life in Measurable Terms

Life can be defined in many ways, but for our purposes, the definition is of use only if the various parts of the definition can be detected and quantified to some degree. For this reason, we have listed the following features of life that we feel are of the most use for our search (Nealson and Conrad, 1999):

- Structure: Life is a machine designed to convert physical or chemical energy to a biologically useful form -- to accomplish this end, some kind of "hardware" for energy conversion is needed (e.g., life has structure).

- Unique chemistry associated with its structure: For Earthly life, this is a carbon-based chemistry with an elemental ratio that is easily recognizable and distinguishable from

the Earth's crustal abundance, and from minerals and concretions of abiotic origin. The *nature* of chemistry is less important (for our definition) than the *uniqueness* of it.

- High-fidelity replication: Copies of complex structures and the molecules that comprise them are made routinely as a part of the life process, and in fact are part of the definition of success of any given group of biota. We proceed from a few copies of many different molecules to many copies of (often very complex) molecules of life.

- Evolution: Life makes a sufficient number of mistakes during replication that some variability is built into the system, thus allowing for biological evolution of chemistry, structure, and behavior.

- Energy consumption and product excretion: It is a fundamental feature of life that energy flows through it. Energy is taken from the environment to make the complex chemical structures of which it is composed. It also creates metabolic products as a result of the energy consumption. In many cases, it is possible to recognize life by gradients of reactants and products produced during growth and metabolism, which can be found in the fossil record.

- Deliberate movement: Life must develop some means for escaping from its own metabolic end products and/or finding nutrients as its own chemical abilities evolve and improve. Perhaps one of the first innovations of life would thus be motility, although as life becomes abundant, specific symbioses may be used to achieve the same ends.

Structure

Structure is one of the most easily identifiable and useful measurable parameters of life. Typically, we define a structure by its function—its role as "hardware." A role is hard to measure, so a way to address this complication is to define structure is as the way in which some chemicals are arranged in three dimensions. One of the great challenges of looking for life will be the strategy whereby it can be found in the case where it is not abundant. By beginning with the search for structures, one can effectively reduce the search space, using methods of pattern detection and distinction. Once structures are identified, then a number of techniques can be used to probe the region of interest to determine its chemical composition and character. For living organisms, structures should be rather easy to find, as the density of living things is usually quite distinct from that of the rocks or soils on which life is found. However, as fossils form, the distinctions may blur. It is thought that only a relatively small percentage of former life is preserved as fossils.

The explanation traditionally offered is that the conditions necessary for preservation are rare: rapid burial, resistance to chemical and biological attack, and so forth. We expand the definition of fossil beyond molds, casts, preservation in amber, permineralization, and traces, such as burrows, trails, tracks, etc., to include a class of chemical fossils--

collections of elements, isotopes, etc., differentiable from host rock. These chemical fossils may be too diffuse to recognize as a conventional fossil, that is, they might present varying degrees of parameter contrast with the host rock, depending upon degree of preservation. Contacts could be sharp or gradational, not unlike using a crayon to color "inside the lines," or to color "outside the lines, " or in the least distinct case, to color without any lines at all, where the color itself defines the shape.

Chemical Composition

By chemical composition, we mean several possibilities, such as elemental abundance, degree of metallicity, charge state distribution of metals, stable isotopic fractionation, presence and type of organic compounds, etc. The necessary detection limits, precision, accuracy, sampling rate and other specifications for detection of these chemical features is dependent upon the differences in magnitude and scale between the region of interest and the surrounding background. One might think of this as akin to the signal-to-noise (S/N) ratio in an electronic or optical device. Obviously, a large S/N ratio is desirable, and different spatial scales may yield varying degrees of contrast between signal and noise. This implies that life may be harder to detect in some environmental media than in others, depending upon the physical states of the environment, e.g., sediment, water, vapor, solid rock, etc. One might also infer that the required S/N ratio for the detection of extant life may well be different from that for extinct life, if for no other reason than that extinct life is not well preserved in air or water. Such an inference might further lead one to constrain the search space to crustal planetary material when life is not readily observable in an atmosphere or hydrosphere. On Earth, the endolithic lifestyle is fairly common, that is, rocks host both living communities and fossils.

Biosignatures can be atmospheric, hydrospheric, or lithospheric (Figure 2) and may be the direct detection of life itself (e.g., the signature is the organism) or the detection of some "footprint in the sand" left by a life form. Indeed, the discovery of the footprints left by the Apollo astronauts on the moon, the abandoned Pathfinder lander on Mars, or a planetary atmosphere with a disequilibrium mixture of gases due to biogenic input or consumption-- these would all be causes for excitement to those involved in the search for life. Each carries a message that says, "return to this place and do more measurements and studies." It is this message that is the essence of our strategy.

For this report we focus on biosignatures that might be obtained from organisms or their remnants in geological materials. Again, one must remember that the signals can be of many sizes, structures and compositions. For the detection of life or its fossils we move to the discussion of structures and compositions.

There are distinct patterns of elemental abundance from stellar spectral data, various meteorite types, rocks from the Earth's mantle, crustal rock types and living organisms (Figure 3). These data, obtained with several different spectroscopic methods can be normalized to chondritic values, as is consistent with the geochemical literature. This type of elemental inventory and comparison leads one to infer that it is often the minor and trace elements that distinguish life from its host environment. The process of

planetary differentiation affects the abundance of available elements at the Earth's surface. Oxygen is the dominant element in the Earth's crust, 50% by weight and, at surface temperatures, many other abundant elements combine with it more readily that with any other element. The result of this strong affinity is that the bulk of crustal materials are silicates and carbonates, and the partitioning of metals into these phases affects their biological availability (Williams and Frausto da Silva, 1996). In addition, the solubility of elements and their ionic activities in water, bot marine and fresh, are also important factors in determining what elements will partition preferentially into life (ref).

It is not the specific abundance(s) of an element or several elements, but the association of elements that may lead one to suspect a biosignature, although the presence or concentration of geochemical reaction products at levels that are inconsistent with abiotic reaction rates may be suspicious. The most important consideration with regard to elemental distribution as a candidate biosignature is that the distribution be inconsistent with the surrounding rock, or not be predicted by thermodynamic phase equilibria. This context requirement is also true for other types of chemical biosignatures, such as the distribution of oxidation states of metals throughout a material, the fractionation of stable isotopes, or the tendency toward enantiomeric purity (chiral preference) in some biologically important organic compounds such as amino acids and sugars. Again, environmental context is the key.

Measurement Methods

There are many tools available for the measurement of chemical composition in solids, liquids and gases, and this discussion is not meant to be a complete inventory of techniques. We are focusing on inorganic chemical biosignatures, in specific, elemental abundance and distribution. But the principles of our protocol would apply to the analysis of organic biosignatures as well.

In evaluating life detection methods, what one quickly realizes is that there are several considerations that challenge the appropriateness of any single technique, and that a suite of instruments may be required for life detection, particularly in the field. This is, in part, because of the multi-scalar aspect of chemical biosignatures. No single technique will have the capability to analyze samples ranging several orders of magnitude in size or requiring sampling rates of equal variability. When looking for life in situ, the portability of instruments becomes an issue. The degree of sample preparation required is also important, not only because of the technical challenge of sample preparation in the field, but also because some sample preparation methods that require chemical extractions, for example, introduce statistical uncertainties over the efficiency of the preparation.

The strategy of the hunt must include a survey step-- a fairly rapid scan of a large spatial region, using a minimally invasive technique. Exactly how large a spatial region should be surveyed is also worthy of discussion. When looking for distant planets that may be habitable, the techniques presently under development are optical interferometric ones. We may have only two pixels of data from which to characterize the potential habitability of world. At the other extreme with respect to size, the interface between an endolithic (rock-dwelling) bacterial cell wall and host rock may exhibit features on the order of a

few hundred Ångstroms, for which we would need to use a transmission electron microscope (TEM) or atom probe. These techniques, however, require substantial sample preparation. For transmission to be possible, the sample must be very thin. Clearly, such techniques would not be suitable for surveying large areas in an Earthly environment, areas on the scale of meters or even kilometers. We select these scales because, as field investigators, these are the scales of observation we would need to tackle on an expedition to various cryptic habitats on our own world. Optical techniques such as infra red imaging and scanning spectroscopy (absorbance and reflectance) would be useful at these scales-- portable devices exist with sufficient spectral resolution to narrow down the region of interest to say, a square meter. At that point, visible imaging and broadband or laser-induced fluorescence imaging could be used to scan the region for further areal constraint before deploying other tools which can probe a sample with the resolution of a few millimeters to tens of micrometers, such as vibrational spectroscopy.

The environmental scanning electron microscope (ESEM), though presently not field portable, requires no sample prep other than cutting the specimen down to a thumbnail size. Even wet samples can be probed for their elemental inventory using an energy dispersive x-ray fluorescence technique. Field-worthy techniques for conducting elemental analysis at the same spatial scales (~100nm to a couple of mm)) are somewhat more destructive, for example laser-induced breakdown spectroscopy.

High-energy probes, that is techniques that analyze samples with electrons, protons, neutrons, x-ray photons, gamma rays, or coherent light of various wavelengths, can search over various spectral bandwidths and spatially resolve data from a few tens of nanometers to several millimeters. Some of these techniques have already become portable and can be further miniaturized. Others require the energy of a synchrotron light source or other particle accelerator (Table 1).

As we test different life detection technology, we will become more familiar with the character of various biosignatures, and this will, in turn, lead to more targeted technology with respect to sample preparation, spatial and spectral resolutions, etc.

The compilation of a biosignature database requires the co-development of an abiosignature database organized by environment, habitable or not. On this planet, almost every lithospheric or hydrospheric environment is, to some degree, habitable by some organism or other. But the relative impact of resident organisms on the chemistry and structures of an environment is dependent upon several factors, including how many organisms are present. So it is important that we learn how to distinguish between the biological and the non-biological contributions to the chemistry and structure of an environment. This differentiation can, in part be assisted by principal component analysis (ref--Michael? or Evan?)

The "taxonomy" of biosignatures is a matrix and the unique set of signatures that describe any given type of life are tensors of high orders. In much the same way that the several physical properties of minerals can be used in their identification as unique, inorganic crystalline solids, we seek to integrate several physical properties of life to

uniquely distinguish it from non-life (Table 2). There are both types and scales of biosignatures that will or will not correlate to one another in a statistically meaningful way, and by gathering large numbers of such biosignatures in Earth environments, we begin the process of learning which of these correlations can be useful for clarifying life detection data.

Images and Complexity

We humans are very visual creatures. We tend to use our eyes as triage instruments, supporting our first impressions with consensus from our other senses. But whether we are *looking* for life, *listening* for life, or detecting it by any other means, for that matter, we must build measurable increments into the observation process, just as we are requiring measurable data. This incremental, or digital measurement process is what allows us to extend our senses with devices, not just spatially (in terms of distance), but also in terms of *sensitivity* and *sampling rate*, or time increments. What this means, specifically, is that when we look at an image of a living organism, there has to be some way of quantifying how we recognize the image as a living thing. We take the approach that astronomers use when they analyze images of distant galaxies (Michael's references). Using computers, we analyze regions of images for a quality called *complexity*, which can be defined as the amount of information in a given pixel or group of pixels of an image. Algorithms have been developed for recognizing complexity by determining the data compressibility of an image. More incompressible images are more complex and compressible images are considered less complex. It is important to understand that the complexity of an image may be observable only at some wavelengths and not at others. This supports our earlier assertion that no one analysis or analytical tool is sufficient for proving the observation of life.

SUMMARY

The detection of life, though daunting, can be regarded as a process, the steps of which can be individually and recursively addressed. By avoiding Earth-centric criteria, biosignatures can be defined in such a way that they are less likely to be missed in an alien environment. The repertoire of life detection techniques must include both rapid scans over large spatial areas and high-resolution analysis of small spatial areas.

Biosignatures are present over various spatial and temporal scales. Time resolved studies are sometimes necessary to observe disequilibrium phenomena, as life can maintain chemistry that is inconsistent with thermodynamic equilibrium. Rates of chemical diffusion may be insufficient to explain the presence or concentration of reaction products that would be kinetically impeded in abiotic geochemistry.

The acquisition of candidate biosignature measurements is only part of the challenge of life detection; the assurance of statistical meaning for diverse data sets at disparate scales is, itself, an important part of the process. Principal component analysis can be used to assist the investigator in classifying observations as life, past life, or non-life.

Although we are still far away from the robot who can tell us, "Danger! Alien life form approaching...," we are beginning to understand what the ideal instruments might measure, what the accuracy and precision requirements are, and how we might lend statistical credence to such measurements. Life, however much an enigma, will become ever more recognizable as we learn to think of it in terms of what is measurable about it. When we can identify life and never miss it on Earth, we will be ready to take our show on the road.

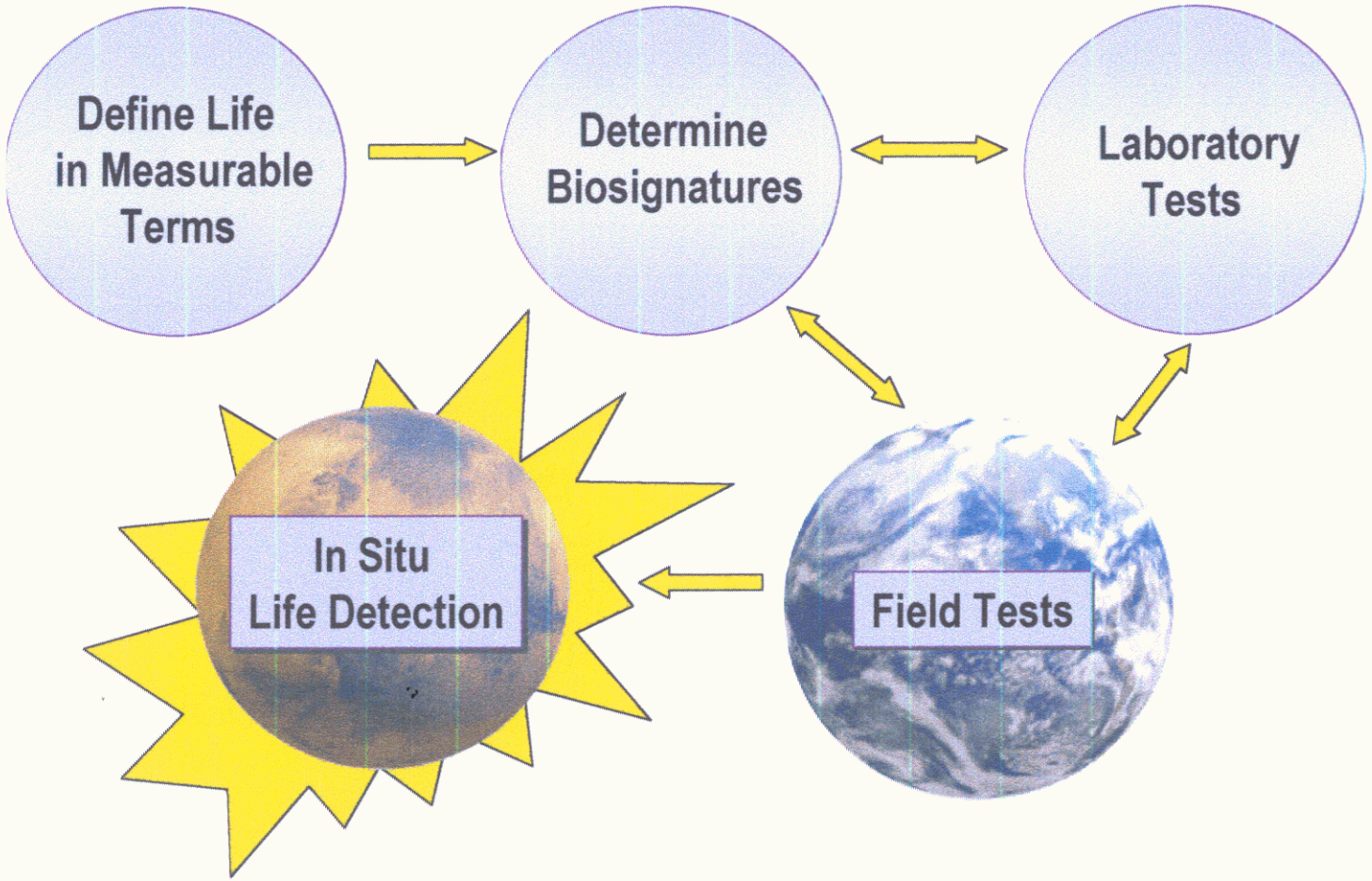
Define Life in Measurable Terms
Determine Biosignatures
Laboratory Tests
In Situ Life Detection
Field Tests

Figure 1

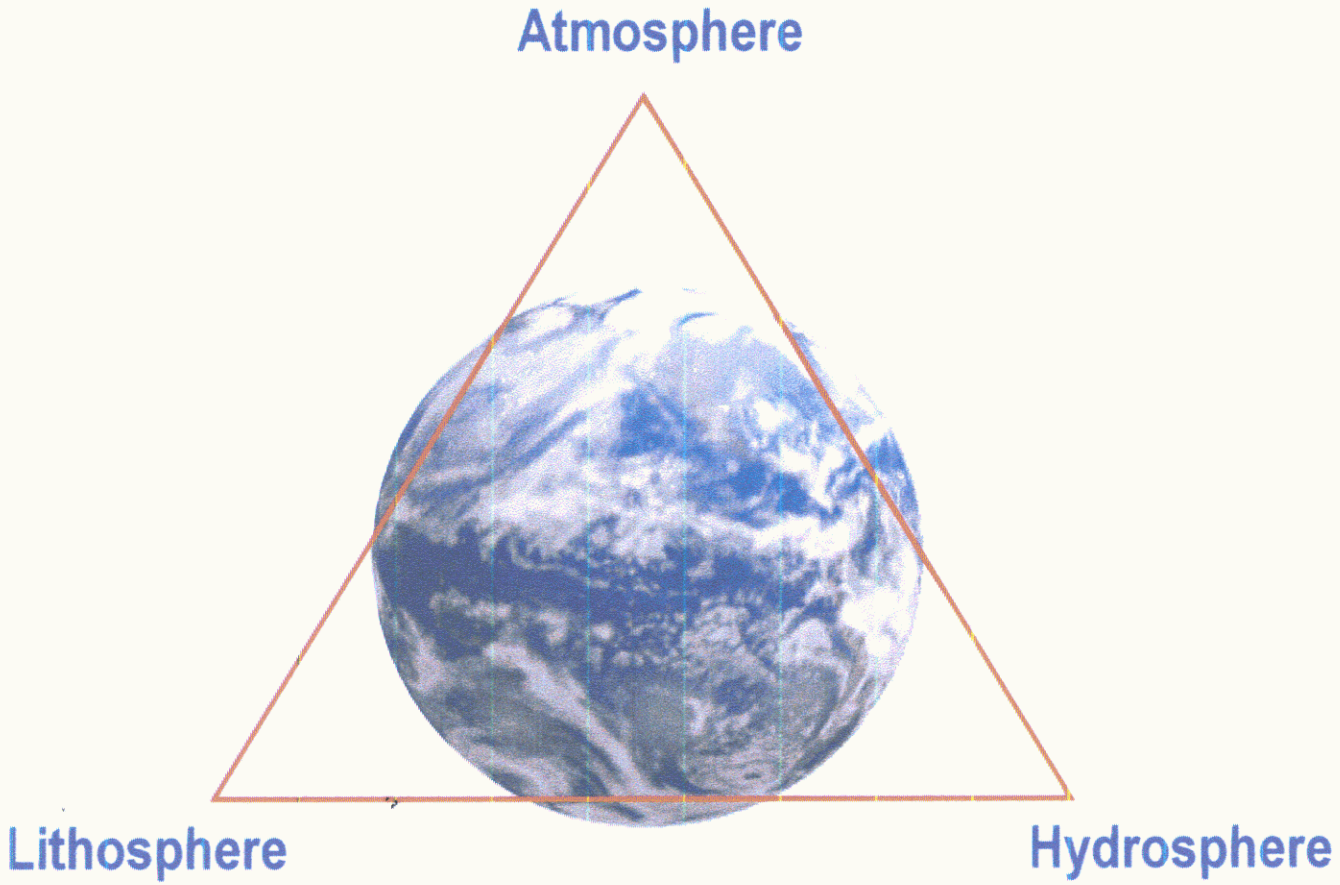

Figure 2

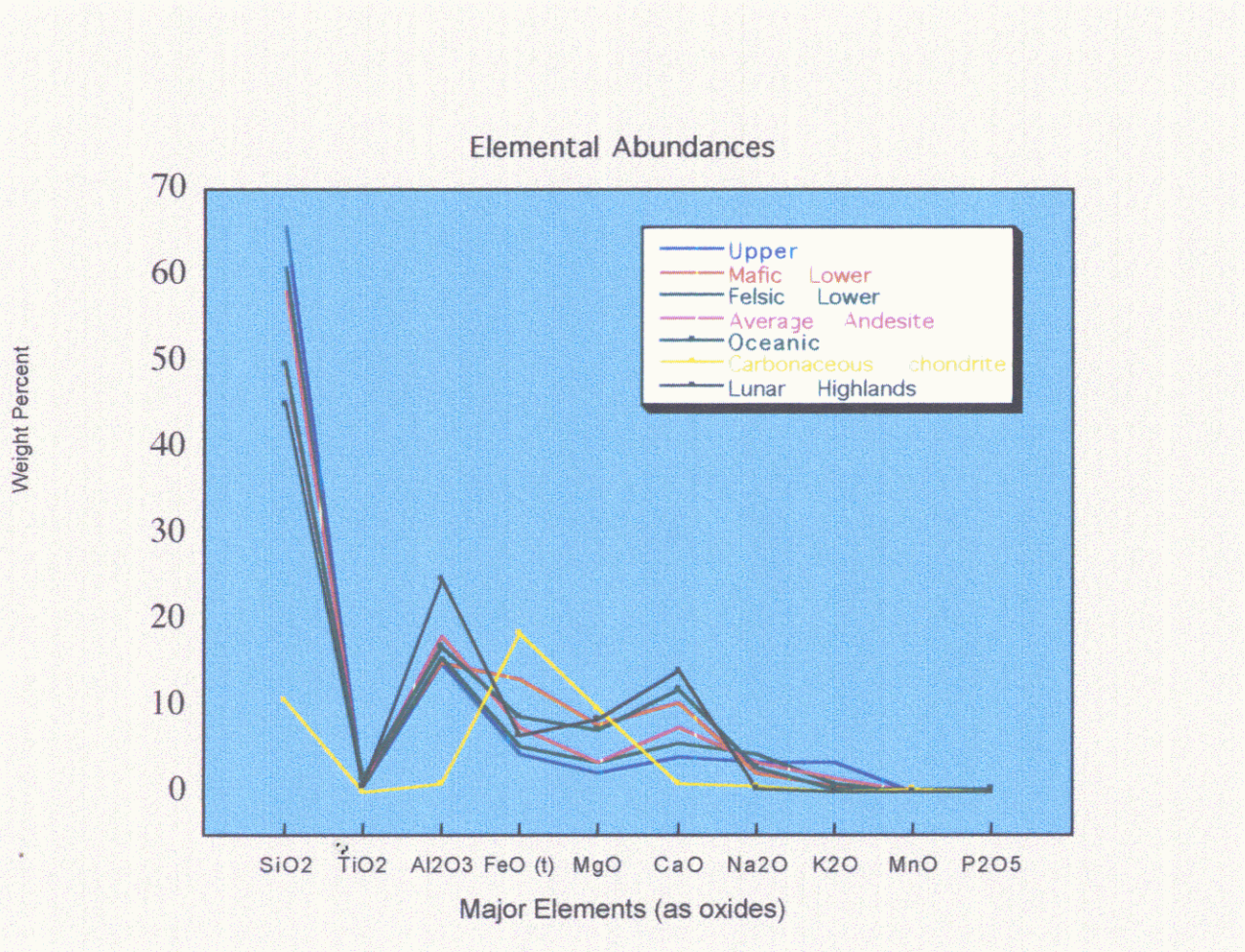
Elemental Abundances
Weight Percent
70
60
50
40
30
20
10
0
Upper
Mafic Lower
Felsic Lower
Average Andesite
Oceanic
Carbonaceous chondrite
Lunar Highlands
SiO2
TiO2
Al2O3
FeO (t)
MgO
CaO
Na2O
K2O
MnO
P2O5
Major Elements (as oxides)

Figure 3