

Development of New Technologies for Removal of Arsenic from Water Supplies Through Biomimicry of Natural Systems

Final Report

by

Jay R. Leverone, Ph.D.
Mote Marine Laboratory
1600 Ken Thompson Parkway
Sarasota, FL 34236

to

Diane Andrews
Economic Development Corporation
of Sarasota County
2601 Cattlemen Road, Suite 201
Sarasota, FL 34232

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Phase I: Synthesis of Existing Knowledge on the Ability of Living Organisms to Remove Arsenic from Ambient Water

“In the twentieth century, product innovations tended to spring from physics. But in the new century, biology may be the central source of innovation. From genomics to biomimicry, the study of life promises to change what companies sell and even how they operate....Biomimicry is not simply using natural organisms for our benefit, but rather applying methods found in natural systems to optimize existing processes and enhance future designs.” (37, 31)

Executive Summary

This project was undertaken in response to a request from the Economic Development Corporation of Sarasota County, which was interested in searching for potential solutions to the challenging problem of removing arsenic from water supplies. This particular problem was raised by a local industry spokesman as a potential area for economic development. Currently, removing arsenic from groundwater is a very costly and difficult process. The primary goal of this project is to search the existing scientific literature to identify organisms that display the ability to remove arsenic from their surroundings. Following this literature search, a synthesis is presented on how these organisms accomplish this task. Finally, this information is discussed in light of current or new technologies that could be developed through biomimicry; that is, the man-made mimicking of these natural designs.

Several unique organisms have been the subject of comprehensive studies regarding their ability to remove arsenic from the environment. This information is summarized in the following report. More interestingly, however, is the fact that most (if not all) organisms, from bacteria to mammals, have the ability to remove arsenic from the environment and take it into their cells. In general, this is accomplished by a unique and complex transport mechanism embedded in the cell membrane.

The most promising avenue for addressing the issue of arsenic removal from water may lie in the ability to “replicate” this biological system and incorporate the active

compound into a bioengineered membrane. Two researchers who are leading the way in the engineering of biomembranes were identified and a summary of their findings and their contact information are provided. This material is highly technical and beyond the scope of this report. It is not even certain that a membrane filtration system that incorporates a bioengineered arsenic-removing complex is possible, or if such a membrane would be effective or efficient in reducing arsenic levels in a full-scale water purification system. However, if biomimicry is to be considered in addressing this particular problem, this type of system theoretically offers the best path to pursue.

Introduction

Novel solutions to technical problems may be found by turning our attention to natural systems. Humans have always looked to nature for inspiration in designing new technologies, and material design on the *molecular* level is no different. Just as the discovery of new drugs from living organisms has the attention of pharmaceutical companies, animal and plant species may provide insight into unique mechanisms for removing heavy metals from the surrounding environment. By studying these natural solutions, engineers could mimic the techniques and develop improved water purification systems based on the lessons learned from these natural systems. This field of study is referred to as biomimicry.

The element arsenic is often found in water supplies and can be toxic to living organisms, including humans. Removal of arsenic from drinking water is a difficult and expensive process using current technology, but many living organisms appear to have the natural ability to isolate arsenic from water. Therefore, a search for a biomimetic solution to the problem of removing arsenic from drinking water is warranted.

The first step in this process is to conduct a thorough search of the scientific literature to identify organisms that have developed the capacity to remove arsenic from their surroundings. Consequently, the goal of this literature search is to identify these organisms and summarize what is currently known about the processes involved in their ability to remove arsenic. This knowledge might then be adapted to water purification processes, specifically with regard to removing arsenic from drinking water.

Arsenic Uptake

Arsenic enters the biosphere primarily by leaching from geological formations (1). Anthropogenic sources include arsenical-containing fungicides, pesticides and herbicides. The human health effects of environmental arsenic exposure can be devastating, and, over a period of decades, can progress to various forms of cancer. Its ubiquity in the environment has led to the evolution of arsenic defense mechanisms in every organism studied, from the bacteria such as *Escherichia coli* to humans (2). As described below, organisms take up As(V) via phosphate transporters (3,4) and As(III) by aquaglyceroporins (5-7). As(V) is reduced to As(III) (8, 9), which is either extruded from cells or sequestered in intracellular compartments, either as free arsenite or as conjugates with GSH or other thiols (10,11). In addition, arsenic can be methylated (12), although this process may increase arsenic toxicity rather than contributing toward detoxification (13).

Arsenic Speciation and Toxicity

Arsenic is a semi-metal or metalloid with two biologically important oxidation states, As(V) and As(III), as the oxyacids arsenic acid (H_3AsO_4) or arsenous acid, also called arsenic trioxide (As_2O_3). In solution at neutral pH, arsenic acid exists as the arsenate oxyanion. The pKa of arsenous acid is 9.2, so that, at neutral pH, it would be primarily present in solution as neutral $\text{As}(\text{OH})_3$. This difference in pKa is relevant for the type of transport system that catalyzes uptake of the pentavalent and trivalent forms of arsenic. The other aspect of arsenic chemistry relevant to biological activity is reactivity of As(III) as a soft metal ion, forming strong bonds with functional groups such as the thiolates of cysteine residues and the imidazolium nitrogens of histidine residues.

In marine environments, arsenic toxicity is a concern. Inorganic arsenate As(V) and arsenite As(III) are the most toxic forms. Briefly, arsenate toxicity occurs when this element replaces phosphate during the oxidative phosphorylation processes. This leads to mitochondrial impairment and inhibition of glycolytic energy metabolism. Methylated

compounds such as methylarsonate (MMA), dimethylarsinate (DMA), trimethylarsine oxide (TMAO) and tetramethylarsonium (TETRA) are considered moderately toxic, while more complex organoarsenic compounds like arsenobetaines (AsB), arsenocholines (AsC) and arsenosugars (AsS) are not harmful.

Arsenic toxicity is strictly related to its chemical form and plants and animals are known to accumulate this element mostly as nontoxic organoarsenic molecules. Contrasting with this general trend, the presence of moderately to highly toxic arsenic compounds recently has been reported in some species of marine worms, showing a completely different profile of represented chemical species. These worms have been observed to accumulate high levels of the relatively more toxic forms of arsenic in specific tissues.

Arsenic Detoxification

All living organisms have systems for arsenic detoxification (see Figure 1). There are two phosphate transporters in the prokaryote *E. coli* that can catalyze arsenate uptake (4, 14). The eukaryote *Saccharomyces cerevisiae* also has several phosphate transporters which participate in arsenate uptake (3, 15). It can be assumed that arsenate is taken up similarly in mammals, although this has not been demonstrated.

One of the first pathways to be described for the cellular uptake of arsenite from *E. coli* indentified a trivalent metalloid transporter, the glycerol facilitator (GlpF) as a likely As(III) transporter (5). GlpF is an aquaglyceroporin, which is a member of the aquaporin superfamily. Aquaglyceroporins are multifunctional channels that transport neutral organic solutes such as glycerol and urea (16). Fps1p, the yeast homolog of GlpF, was recently shown to be the route of uptake of arsenite in *S. cerevisiae* (6).

Mammalian aquaglyceroporins have been shown to catalyze the uptake of trivalent metalloids (7). A strain of *S. cerevisiae* with deletions in the genes for the two arsenite extrusion transporters, Acr3p and Ycf1p (10), was constructed as described below. A strain with disruptions of both ACR3 and YCF1 was hypersensitive to arsenite. Another strain, this one with the triple deletion of ACR3, YCF1 and FPS1, was unable to take up arsenite, which resulted in arsenite resistance.

To examine the arsenite transport properties of mammalian aquaglyceroporins, the genes for the rat AQP9 and mouse AQP7 were cloned into a yeast vector and their ability to complement the arsenite-resistant phenotype of the triple yeast mutant was tested. AQP9 was expressed well and restored arsenite sensitivity. Cells expressing AQP9 transported both $^{73}\text{As(III)}$ and $^{125}\text{Sb(III)}$, thus demonstrating that this protein can transport metalloids. AQP7 was not expressed in yeast. However, when either AQP7 or AQP9 cRNA was microinjected into *Xenopus laevis* oocytes, increased transport of $^{73}\text{As(III)}$ was observed (7). Human AQP3, AQP9 and AQP10 have been expressed in yeast, although only human AQP9 complemented the arsenite phenotype suggesting that only some aquaglyceroporins transport As(III).

What chemical species of As(III) do the aquaglyceroporins recognize? With a pKa of 9.2, As(OH)_3 would predominate in solution at neutral pH. Since AQP7 and AQP9 conduct transmembrane movement of neutral species, As(OH)_3 , which might be considered an inorganic equivalent of glycerol, is the likely substrate.

Arsenate Reductases

When arsenate is taken up, it is reduced to As(III) prior to being extruded or sequestered. Three independently evolved families of arsenate reductase enzymes have been recognized (20) (Figure 2B). The first sequence of an arsenate reductase is the product of the last gene of the *ars* operon of *E. coli* plasmid R773 (21). Homologues of this *arsC* gene are found in many bacteria, both on plasmids and in chromosomes (1). The crystal structure of the 16 kDa R773 *arsC* has been reported with bound substrate (arsenate) and product (arsenite) (22). In the reaction cycle, arsenate first binds to the anion site that consists of three basic residues, Arg60, Arg94 and Arg107. Phosphate and sulfate compete for binding at this site. In the next step, arsenate (but not phosphate or sulfate) forms a covalent arsenate thioester intermediate with the active site Cys12. It is then reduced in two steps by glutaredoxin and glutathione, producing the Cys12-S-As(III) intermediate, which hydrolyzes to release arsenite. *E. coli* has three glutaredoxins, Grx1, Grx2 and Grx3, any one of which will serve as source of reducing potential for arsenate reduction, although Grx2 is preferred (23).

A second family of arsenate reductases also widely distributed in bacteria is typified by the *arsC* gene product of *Staphylococcus aureus* plasmid pI258 (8) (Figure 2C). It is unfortunate that this enzyme is also called ArsC considering that it is unrelated to the first family of arsenate reductases. The pI258 enzyme uses thioredoxin as the source of reducing potential (24) and has two intramolecular cysteine residues that participate in the catalytic cycle (25). The crystal structures of the pI258 ArsC and its homolog from *Bacillus subtilis* have recently been reported (26,27). Interestingly, this enzyme is related to low molecular-weight protein tyrosine phosphate phosphatases and exhibits low-level phosphatase activity (26).

The third family of arsenate reductases is also related to protein tyrosine phosphate phosphatases, although to a different family of phosphatases than the pI258 ArsC. The only member of the family to be characterized to date is Acr2p from *S. cerevisiae* (28,29). It is related to the superfamily of protein phosphatases that includes CDC25a, a cell cycle phosphatase (30) and uses a similar HisCys(X)5Arg motif in its active site (32). Like the R773 ArsC, Acr2p has a single active site cysteine residue and uses glutaredoxin and glutathione as reductants (32). Even though it is not related to either of the bacterial ArsC arsenate reductases, it can be heterologously expressed in *E. coli* and complements an *arsC* deletion. It does not exhibit phosphatase activity.

The ease by which a reductase can be changed into a phosphatase suggests that, under the selective pressure of ubiquitous arsenate in the environment, arsenate reductases evolved from phosphatases. No mammalian members of any of the three families of arsenate reductases has been identified to date. However, the relatedness of Acr2p to the widespread protein tyrosine phosphatase family makes it difficult to identify homologs with arsenate reductase activity through a genomics approach. Arsenate reductase activity has been observed *in vitro* in extracts from human liver (33). Recently the activity has been attributed to the enzyme purine nucleotide phosphorylase (34). Whether this enzyme functions *in vivo* in arsenic detoxification has not yet been examined.

Cytosolic arsenite, whether as the product of arsenate reductase or from uptake via an aquaglyceroporin, is detoxified by removal from the cytosol (1,35). It is rather counterintuitive that arsenate, which is not very toxic, should be converted to the more

toxic arsenite prior to transport out of the cytosol. It is perhaps accidental that there are no arsenate-specific exclusion systems in nature.

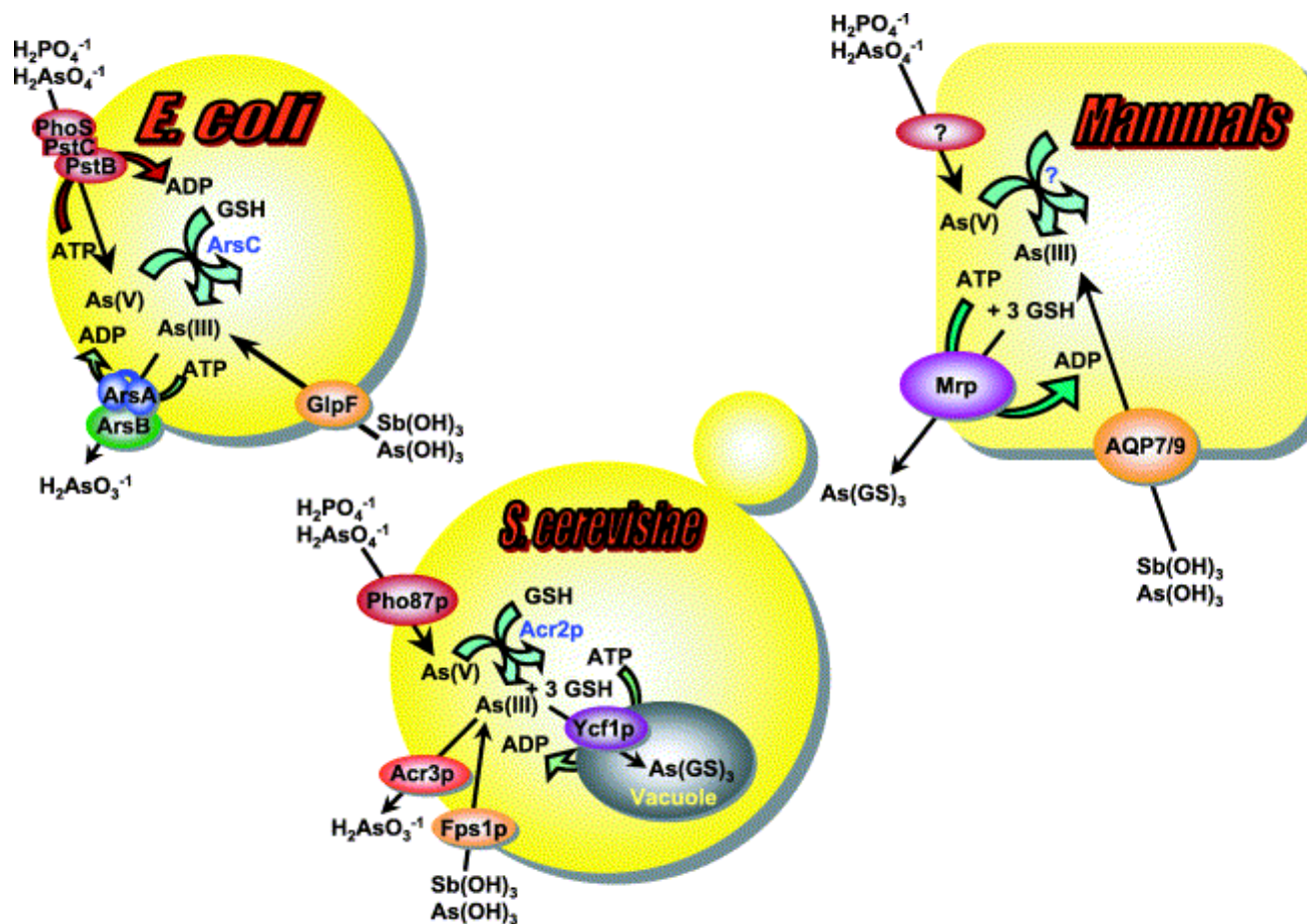


Figure 1. Arsenical detoxification in prokaryotes and eukaryotes. Arsenate ($\text{As}^{(V)}$) is taken up by phosphate transporters, and $\text{As}^{(III)}$ is taken up by aquaglyceroporins (GlpF in *E. coli*, Fps1p in yeast and Aqp7 and Aqp9 in mammals). In both *E. coli* and *S. cerevisiae*, arsenate is reduced to arsenite by the bacterial ArsC or yeast Acr2p enzymes. In both organisms, glutathione and glutaredoxin serve as the source of reducing potential. The proteins responsible for arsenate uptake and reduction in mammals have not yet been identified. In *E. coli*, arsenite is extruded from the cells by ArsB alone or by the ArsAB ATPase. In yeast Acr3p is a plasma membrane arsenite efflux protein, and Ycf1p, which is a member of the MRP family of the ABC superfamily of drug-resistance pumps, transports $\text{As}(\text{GS})_3$ into the vacuole. In mammals Mrp isoforms pump $\text{As}(\text{GS})_3$ out of cells. For example, Mrp2 extrudes $\text{As}^{(III)}$ into bile (From 1a).

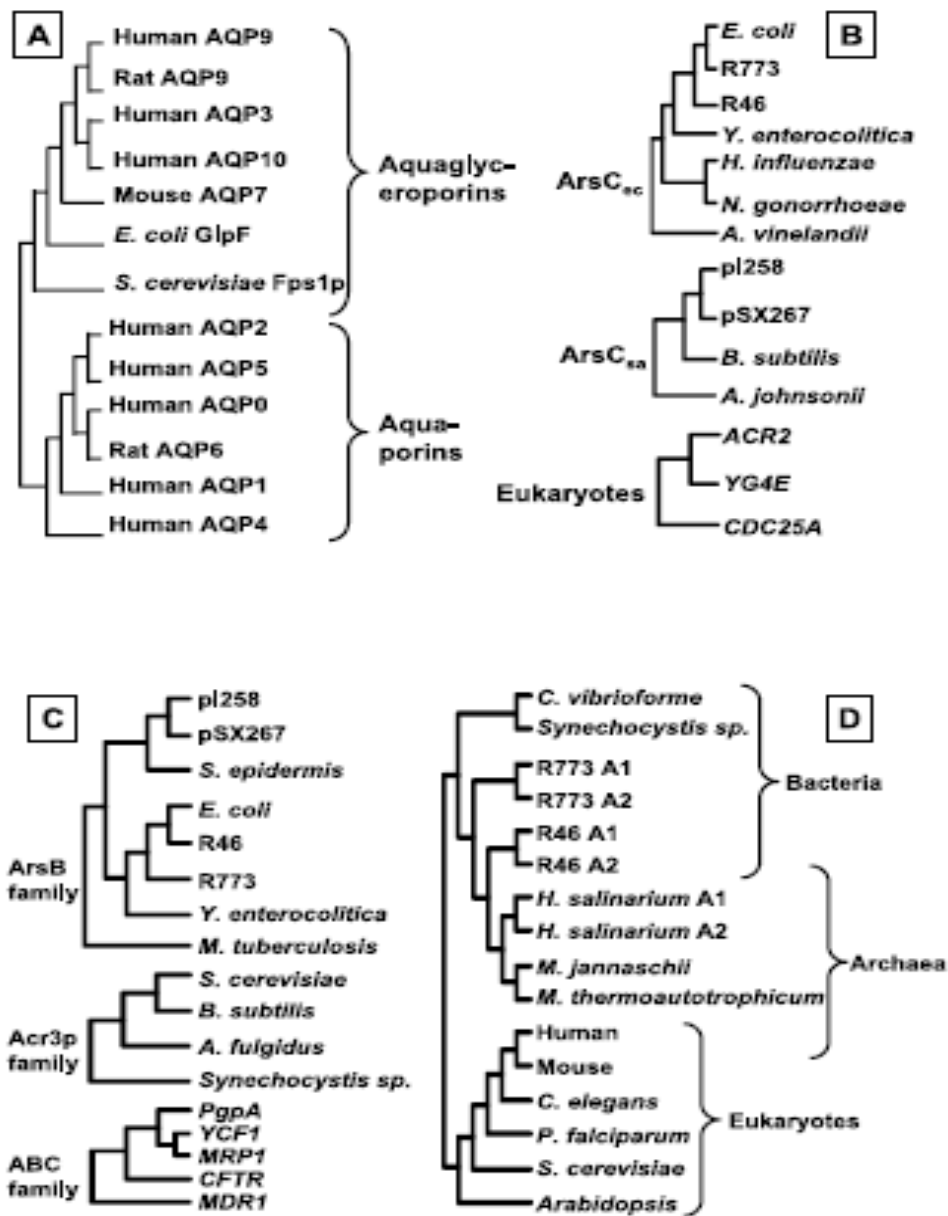


Figure 2. Families of arsenic detoxification proteins (From 1a). A: The aquaporin superfamily. There are two subfamilies of aquaporins [16]. The true aquaporins such as AQP1 allow only water through the channel. The aquaglyceroporins have larger pores and allow uncharged solutes such as glycerol and urea through. $\text{As}(\text{OH})_3$ enters cells by members of this subfamily. B: Three families of arsenate reductases. Reductases involved in arsenate detoxification have apparently evolved independently at least three times [20]. C: Three families of arsenite transporters. The ArsB family of arsenite uniporters has been so far identified only in bacteria [1]. The Acr3p family was first identified in the *B. subtilis* SKIN element, but members were subsequently found in yeast and archaea. The ABC superfamily of solute-translocating ATPases includes the Mrp family. D: The ArsA family.

Arsenic Accumulating (Bioindicator) Organisms

The earliest reference to the search for organisms that could remove arsenic from the aqueous phase involved a preliminary screening of freshwater algae having high resistance to inorganic arsenic (42). Since then, several organisms have been identified with this special capability and, subsequently, have been the subject of intense research. The most commonly studied organisms, their biology and ecology, and what is currently known of their arsenic removal capabilities, are summarized below.

The Chinese brake fern, *Pteris vittata*

The concept of phytoremediation has been developed since 1980. This biotechnology uses plants for environmental restoration and has become an emerging cleanup technology. Metal-tolerating plants, including hyperaccumulators, are desirable for stabilizing mining tailings and extracting metals from contaminated soils.

The Chinese brake fern (*Pteris vittata*) was discovered growing on a site in central Florida that had been contaminated with chromated copper arsenate (46). This plant, considered the first known arsenic hyperaccumulator as well as the first fern found to function as a hyperaccumulator, is extremely efficient in extracting arsenic from soils and translocating it into its above-ground biomass, namely the fronds. Since its discovery, this plant has been the focus of intense research on arsenic accumulation.

One of the earliest studies investigated the interactions of arsenate and phosphate (which are chemical analogues) on arsenic uptake, distribution and speciation (40). Increasing phosphate sharply decreased arsenic uptake, with the effect being greater in the roots than the fronds. Increasing arsenate supply decreased phosphorus in the roots, but not in the fronds. Presence of phosphate in the uptake solution decreased arsenate influx markedly, whereas phosphorus starvation increased the maximum net influx by 2.5-fold. The rate of arsenite uptake was 10% of that for arsenate in the absence of phosphate. Neither phosphorus starvation nor the presence of phosphate affected arsenite uptake. From this research it was concluded that arsenate is taken up by *P. vittata* via the phosphate transporters, reduced to arsenite, and

sequestered in the fronds primarily as arsenite. So far, only inorganic arsenite and arsenate have been found in Chinese brake fern. Organoarsenic compounds have not been detected.

The efficiency with which arsenic is absorbed by the excised pinnae of the Chinese brake fern led to the suggestion that excised plants could be used to clean up arsenic from contaminated water by simply floating them in the water (40). Whether anyone has followed up on this claim and investigated this possibility is unknown.

Arsenic absorption results in the formation of thiol, with MMA causing the greatest level of formation. The ability of *P. vittata* to efficiently reduce As(V), facilitated by phosphorus, and to quickly produce thiols might collectively have contributed to the capability of this plant to hyperaccumulate arsenic.

Additionally, mycorrhiza fungi, which are integral, functional parts of plant roots, play an important role in alleviating metal toxicity in plants. The dominant forms of mycorrhizas for symbiosis with plants are arbuscular mycorrhizas. These arbuscular mycorrhiza not only tolerate arsenic, but their presence increase frond dry mass at high arsenic application rates (41). Furthermore, these fungi increase arsenic uptake across a range of phosphorus levels, while phosphorus uptake is generally increased only when there was no arsenic amendment. The indigenous mycorrhizas found at contaminated sites aid their host plants in accumulating arsenic. Additional systematic research on the mechanisms involved in metal absorption and translocation in both mycorrhiza fungi and metal hyperaccumulator plants is needed.

The marine polychaete worm, *Sabella spallanzanii*

The polychaete *Sabella spallanzanii* (Gmelin) (Sabellidae) is a widely distributed species throughout the Mediterranean where it is commonly found in sheltered, shallow areas and up to 30 m depth in more exposed waters. Its sedentary habit, filter-feeding behavior and capability to colonize disturbed environments such as harbors suggest the potential utility of this organism as a bioindicator in environmental monitoring programs. It has also been the subject of intense research into arsenic accumulation and biotransformation. The body of this marine worm is differentiated into two distinct regions: 1) the thorax, which is protected within a muddy tube and remains buried below the sediment surface; and 2) featherlike filaments, called the branchial

crown, which can be extended into the water column for both respiration and filter-feeding activities.

The basal levels of arsenic in both the thorax and branchial crown of *S. spallanzanii* were first characterized in 2002 (43), with particular attention to the various chemical forms and their distribution in different subcellular fractions. Specimens of *S. spallanzanii* were collected from four different locations in the Adriatic and Tyrrhenian Sea. Total arsenic concentrations in tissues of this worm were not different between collection sites, which suggests that the levels measured in this study represent basal levels typical for this species. On the other hand, there was marked variation between the different tissues. The thorax averaged $47.5 \pm 19.1 \mu\text{g/g}$ of arsenic(dry weight), while the branchial crown had a 20-fold higher arsenic concentration with mean values of $1,036 \pm 136 \mu\text{g/g}$. This is among the highest reported arsenic concentration for any marine organism.

As much as 90% of the arsenic was associated with the cytosolic fraction in both the thorax and branchial crown, existing mainly in a soluble form (70%). The predominant chemical form in both tissues was DMA (up to 85% of total content). Another methylated form, TETRA, accounted for about 5%, while more complex and non-toxic organoarsenic compounds (such as AsB and AsC) accounted for less than 10%.

Bioaccumulation rates in this polychaete worm are greater for inorganic than organic forms. These inorganic forms subsequently undergo methylation to DMA and TMAO. Variation in the metabolism of glutathione suggests that this thiol is involved in these methylation processes.

From an ecological standpoint, it has been hypothesized that elevated concentrations of moderately toxic DMA in the branchial crown might act as chemical deterrent to protect these exposed and vulnerable tissues from predation. This was demonstrated in limited feeding trials where fish ate the thorax but rejected the branchial crowns. Arsenic concentrations in this worm also show seasonal variations which mainly reflect changes in DMA and trimethylarsine (TMAO).

The marine polychaete lugworm, *Arenicola marina*

The polychaete *Arenicola marina* is found in shallow waters of the Mediterranean Sea... [describe distribution and habitat]. This worm burrows into the sediment and feeds on detritus and surface sand falling into their funnels. It is exposed to arsenic when it ingests sediment particles and the surrounding water. Inorganic arsenite (As^{III}), which is highly toxic, is by far the major arsenic species in field contaminated *A. marina*, constituting 58% of the extractable, or water soluble arsenic (44). This is the first report of a marine organism containing arsenite in such appreciable amounts (45).

Following arsenate exposure in the laboratory, three of the arsenic species naturally present in *A. marina* showed large increases in concentration: arsenate increased seven-fold, arsenite increased 13-fold, and DMA increased 46-fold. These data are consistent with a pathway involving arsenate reduction and methylation first described in bacteria. Although it could not be verified whether these processes were carried out by the worms themselves or by associated microorganisms, it is more likely that DMA was produced within the worms and not by microbes in the seawater (44).

The polychaete worms, *Nereis diversicolor* and *N. virens*

These two species of *Nereis* polychaete worm accumulate arsenate from seawater and transform it into TETRA, most likely by sequential reduction and oxidative methylation steps via MMA, DMA, and TETRA. Methylation of inorganic arsenic is assumed to be a process of detoxification. Thus, many marine organisms when exposed to arsenate are able to methylate it to less toxic metabolites.

Based on observations that different species have different methylated metabolites when exposed to arsenic, differences among species probably play a larger role than environmental exposure in determining the arsenic compounds present in different organisms.

Possible Technologies, Biomaterials and Processes

“Humans have always looked to nature for design inspiration, and material design on the molecular level is no different. Here we explore how this idea applies to nanoscale biomimicry, specifically examining both recent advances and current work on engineering lipid and polymer membrane systems with cellular processes.” (36)

This quote is from a team of researchers designing and engineering biomimetic nanostructured materials (36). Their research involves packaging proteins in an *in vitro* environment where they can continue to function. One model system involves a coupled bacteriorhodopsin and ATPase in an artificial membrane. Other model systems include liposomes and polymersomes, sol-gel processes and bubble architecture.

These technologies seem to hold the most promise for ultimately developing systems that can selectively remove arsenic (or other heavy metals) from source waters. How this can be accomplished is left for the next phase of this project and should be taken up by a team of professionals who have collective expertise in water purification systems, protein molecular biochemistry and nanotechnology.

A bibliography of conventional methods for arsenic removal in the water purification process is presented as an Appendix. This bibliography should be useful in reviewing the effectiveness of current methodologies and may provide insights regarding the next phase of this project.

Conclusions and Recommendations

The discovery that essentially all living organisms possess the ability to take up arsenic from the environment was surprising; however, this ubiquitous finding may prove advantageous in developing an artificial system which can mimic this process. However, actual technologies that incorporate biomimicry into their designs are, at this time, very limited. There was only one research group that had developed a “biomimetic” material, and this group found the process to be very complex and difficult (36). However, it is the recommendation of this report that the best “route” for developing improved water treatment or purification that would remove arsenic

should follow the processes used by this group, which essentially involve membranes embedded with bioengineered “molecules” that specifically remove arsenic.

There are several questions that must be addressed before actually pursuing the development of this new technology. Some that come to mind are:

1) What would it take, and is it even possible, to create biomolecules with the capability to specifically take up arsenic?

2) Could these artificially manufactured biomolecules be incorporated into a membrane and still retain their function?

3) Even if they can be successfully manufactured, would these new biomimic membranes be capable of removing low levels of arsenic under the settings and conditions of a full-scale water purification system?

To answer these questions and determine if this project should move to the next phase, it would be prudent for the EDC to communicate with this research group and discuss the feasibility of undertaking such a commitment of time, money and resources. Their contact information is provided on the following page.

Professional Expertise

Bionanotechnology of Biomembranes

Drs. David W. Wendell and Jordan Patti
UCLA Department of Bioengineering
20 Westwood Plaza
7523 Boelter Hall
Los Angeles, CA 90095-1600

Dr. Carlo D. Montemagno
College of Engineering
University of Cincinnati
ENGRG-Dean's Office ERC 801
PO Box 210018
Cincinnati, OH 45221

Biochemistry of Arsenic Detoxification

Dr. Barry P. Rosen
Department of Biochemistry and Molecular Biology
Wayne State University, School of Medicine
Detroit, MI 48201

Arsenic and Chinese Brake Ferns

Dr. L.Q. Ma
University of Florida
Soil and Water Science Department
Gainesville FL 326011-0290

(Qingdao University of Science and Technology
Mailbox 87
Qingdao, 266042
P.R. CHINA)

Arsenic and the Polychaete Worm, *Sabella spallanzanii*

Drs. D. Fattorini and F. Regoli
Istituto di Biologia e Genetica
Università Politecnica delle Marche
Ancona. ITALY

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APPENDIX B
Master Journal List

ACS Symposium Series
Analytical and Bioanalytical Chemistry
Applied and Environmental Microbiology
Applied Organometallic Chemistry
Aquatic Toxicology
Archives of Environmental Contamination and Toxicology
Biological Trace Element Research
Birth Defects Research
Bulletin of Environmental Contamination and Toxicology
Canadian Journal of Fisheries and Aquatic Sciences
Cell Biology and Toxicology
CIESM Workshop Monographs
Chemical Ecology
Chemical Geology
Chemosphere
Chinese Science Bulletin
Comparative Biochemistry & Physiology C-Toxicology & Pharmacology
Critical Reviews in Toxicology
Czech Journal of Animal Science
Ecotoxicology
Ecotoxicology and Environmental Safety
Environment International
Environmental Contamination and Toxicology
Environmental and Experimental Botany
Environmental Geochemistry and Health
Environmental Health Perspectives
Environmental Science & Technology
Environmental Toxicology & Chemistry
Environmental Monitoring and Assessment
Environmental Research
Environmental Pollution
Environmental Toxicology
Environmental and Experimental Botany
FEBS Letters
Food and Chemical Toxicology
Fresenius Environmental Bulletin
Geochimica et Cosmochimica Acta
Human and Ecological Risk Assessment
Hydrobiologia
Indian Journal of Environ Health
International Journal of Environmental Analytical Chemistry
International Journal of Environment and Pollution
International Journal of Phytoremediation

International Journal of Systematic and Evolutionary Microbiology
International Microbiology
Journal of Analytical Atomic Spectrometry
Journal of Applied Ichthyology
Journal of Bacteriology
Journal of Dairy Research
Journal of Environmental Monitoring
Journal of Environmental Quality
Journal of Environmental Sciences-China
Journal of Food and Drug Analysis
Journal of Hazardous Materials
Journal of Industrial Microbiology and Biotechnology
Journal of Occupational and Environmental Medicine
Journal of Phycology
Journal of Toxicology and Environmental Health
Marine Environmental Research
Marine Ecology-An Evolutionary Perspective
Marine Pollution Bulletin
Microchimica Acta
Mycorrhiza
Nature
New Phytologist
Plant Biology
Plant & Cell Physiology
Plant and Soil
Reviews in Environmental Health
Science of the Total Environment
Science in China Series C-Life Sciences
Separation Science and Technology
Small
Soil Biology & Biochemistry
Soil Science and Plant Nutrition
Soil Science Society of America Journal
Toxicology
Toxicology and Applied Pharmacology
Toxicology Letters
Trac-Trends in Analytical Chemistry
Water SA
Water Air and Soil Pollution
Water Research

APPENDIX C
Master Authorship List

- Abedin, MJ, Cresser, MS, Meharg, AA, Feldmann, J and Cotter-Howells, J. 2002
Aboal, JR, Fernandez, JA and Carballeira, A. 2004
Adamo, P, Giordano, S, Vingiani, S, Cobianchi, RC and Violante, P. 2003
Allinson, G, Nishikawa, M and Laurenson, LJB. 2002
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APPENDIX D
Traditional Methods for Removing Arsenic
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