CONTAMINATED SOILS, SEDIMENTS, WATER AND ENERGY

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CONTAMINATED SOILS, SEDIMENTS, WATER AND ENERGY

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Foreword

October of 2014 marked an extraordinary event: the 30th time in which the Annual International Conference on Soils, Sediments, Water and Energy was held in lovely Amherst, Massachusetts. Amherst in the fall is a remarkable visual canvas representing foliage in transition from the greenery of spring and summer through the colorful autumn, toward the stoic, chilly winter. In various ways, it reflects our environmental disciplines which undergo cycles wherein subjects rise and fall in relative strategic importance, but all of which contribute to continuing technical progress forward.

Scientists from many disciplines gather in Amherst each October at the Annual Conference, now entering its fourth decade, to present, discuss, and debate ongoing challenges and tools we developed to meet those challenges. When the Conference was born, the Challenger disaster occupied our hearts and minds, Dire Staits, Duran Duran, Madonna, and Wham! headed up the musical charts, and Michael J. Fox was in movie theaters with the first "Back to the Future". This Conference is a unique forum with broad scientific diversity, in 2014 represented by over 200 platform and poster presentations, 9 technical workshops on focused areas of intense interest, and nearly 50 commercial exhibitors of innovative services and products. Every year brings different environmental management tools and useful products, and the newest investigative techniques, or computer data applications, or specialized environmental services are evaluated in scientific presentations, poster sessions, and during intervening social events where much of the "on-the-ground" work occurs. Regulators, students, educators, property managers, myriad scientists and engineers, and others have easy opportunities to combine and recombine to address subjects of interest to them. Collectively, those discussions and technical sessions describe and dissect another year of activity and progress in the identification, assessment and management of complex environmental issues. With nature on colorful display in Amherst, the importance of the Conference is never more apparent. To grasp the reasons that governments, agencies, corporations, and the general public invest their skills, money, time, and experience to environmental understanding and improvement, it is only necessary to look out the window or take a sunny walk across the UMass-Amherst campus.

Several excellent papers from the landmark 30th Annual International Conference on Soils, Sediments, Water and Energy appear in this volume. We are proud to offer it for your review, and we look forward to seeing you in Amherst in October 2015, when we will again spend several lovely days among colorful maples, larches, beeches, and other trees working with

engineers, biologists, planners, regulatory personnel, other scientists, attorneys, and those in related fields who work daily to improve and protect our environmental heritage.

Dr. Clifford Bruell
Dr. Edward Calabrese
Dr. David Ludwig
Dr. Paul Kostecki
Dr. Christopher Teaf

About the Editors

Dr. Paul T. Kostecki's professional career has focused on research, education and training in environmental contamination with an emphasis on human and ecological risk assessment and risk management of soils. His work includes soil ingestion estimates for children and adults; establishment of scientifically sound cleanup levels for soil; bioavailability of soil contaminants; fish as toxicological models for contamination assessment; and assessment and management of petroleum contaminated soils. Dr. Kostecki has developed and conducted over 55 conferences, workshops and courses both nationally and internationally, and has made presentations at over 100 national and international meetings. Since 1985, his conference at the University of Massachusetts Amherst on Contaminated Soils, Sediments and Water has attracted over 10,000 environmental professionals from over 50 countries. Dr. Kostecki has published over 100 articles and reports, co-edited/co-authored 35 Books and secured over $15M in research support.

Dr. Kostecki co-created the Association for Environmental Health and Sciences (AEHS) in 1989 and served as its Executive Director until 2009. In 2009, he established the AEHS Foundation. He helped found Amherst Scientific Publishers and co-created seven peer-reviewed journals: Journal of Soil and Sediment Contamination (1990); Human and Ecological Risk Assessment (1994); Journal of Phytoremediation (1998); Journal of Environmental Forensics (1999); Journal of Children's Health (2003); Non-Linearity Journal (2003); and Journal of Medical Risks (2004). In addition, Dr. Kostecki co-created the International Society for Environmental Forensics in 2002.

Dr. Kostecki served as Vice Provost for Research and Vice Chancellor for Research and Engagement at the University of Massachusetts Amherst from 2003 to 2009. He served as Special Advisor for the Clean Energy China Initiative, Office of the President, University of Massachusetts from 2009–2011. He briefly left the University of Massachusetts Amherst to establish the online education program for Simmons College, Boston, MA (2011 -2012). He is presently Professor Emeritus in the School of Public Health and Health Sciences, University of Massachusetts, Amherst.

Dr. Christopher M. Teaf is a Board-certified toxicologist with broad experience in evaluation of potential effects from chemical exposures related to industrial facilities, agriculture, waste management facilities, power generation, educational institutions, and products in general

commerce. Dr. Teaf has served on the faculty of the Center for Biomedical & Toxicological Research at Florida State University since 1979, and as Director of Toxicology for Hazardous Substance & Waste Management Research since 1985.

Chris' areas of interest include risk assessments under environmental and occupational elements of federal, state or local regulations, risk communication, and development of risk-based targets to guide remedial actions. He has extensive experience in evaluation of environmental fate and potential health effects from petroleum, solvents, metals, pesticides, pharmaceuticals, biological agents (e.g., mold, microbes) and physical agents (e.g., particulates, asbestos. For over 30 years, he has directed or conducted research in environmental and occupational toxicology for the World Health Organization, NATO, U.S. EPA, U.S. Air Force, U.S. Department of Agriculture (USDA), Florida Department of Environmental Protection, Florida Department of Health, Florida Department of Community Affairs, and Agency for Toxic Substances & Disease Registry (ATSDR), among others. He served as Toxicologist for the Florida Landfill Technical Advisory Group and the state Petroleum Technical Advisory Committee. He served on the Florida Governor's Financial and Technical Advisory Committee and was Chair of the Toxic Substances Advisory Council for the Florida Department of Labor. Chris has organized and taught many graduate and undergraduate courses and technical seminars for presentation to universities as well as international, federal, state and local agencies. He has served as Chair of the Dog Island Conservation District since 2004.

Dr. Teaf has served on editorial boards or as peer reviewer for a variety of journals and is Senior Editor for Human Health of the international journal *Human & Ecological Risk Assessment*. In addition to training, research and advisory services to many environmental agencies and private sector firms, he has provided environmental and toxicological services to the U.S. Attorney, Florida State Attorney, and Attorneys General of FL, OK, and WA. Chris has been qualified as an expert in federal and state courts, as well as administrative proceedings, in a number of states regarding toxicology, health risk assessment, and environmental chemistry.

Dr. Edward J. Calabrese is a Professor of Toxicology at the University of Massachusetts, School of Public Health and Health Sciences, Amherst. Dr. Calabrese has researched extensively in the area of host factors affecting susceptibility to pollutants, and is the author of over 750 papers in scholarly journals, as well as more than 10 books, including Principles of Animal Extrapolation; Nutrition and Environmental Health, Vols. I and II; Ecogenetics; Multiple Chemical Interaction; Air Toxics and Risk Assessment; and Biological Effects of Low Level Exposures to Chemical and Radiation. Along with Mark Mattson (NIH) he is a co-editor of the recently published book entitled Hormesis: A Revolution in Biology, Toxicology and Medicine. He has been a member of the U.S. National Academy of Sciences and NATO Countries Safe Drinking Water committees, and on the Board of Scientific Counselors for the Agency for Toxic Substances and Disease Registry (ATSDR). Dr. Calabrese also serves as Chairman of the Biological Effects of Low Level Exposures (BELLE) and as Director of the Northeast Regional Environmental Public Health Center at the University of Massachusetts. Dr. Calabrese was awarded the 2009 Marie Curie Prize for his body of work on hormesis. He was the recipient of the International Society for Cell Communication and Signaling-Springer award for 2010. Dr. Calabrese received an honorary Doctor of Science from McMaster University, Hamilton, Ontario in 2013. Over the past 20 years Professor Calabrese has redirected his research to understanding

the nature of the dose response in the low dose zone and underlying adaptive explanatory mechanisms. Of particular note is that this research has led to important discoveries which indicate that the most fundamental dose response in toxicology and pharmacology is the hormetic-biphasic dose response relationship. These observations are leading to a major transformation in improving drug discovery, development, and in the efficiency of the clinical trial, as well as the scientific foundations for risk assessment and environmental regulation for radiation and chemicals.

Dr. David F. Ludwig is a systems ecologist by training and a risk assessor by trade. He took an undergraduate Bachelor of Science degree from Rutgers University, a Master's in Marine Biology at the Virginia Institute of Marine Sciences, and a PhD in Systems Ecology at the University of Georgia Institute of Ecology. His career linked environmental consulting with university teaching. He worked in academia, the private sector, and for regulatory agencies, a breadth of background the gives him unique perspectives on environmental matters. Dave's career spanned the globe. He worked in mainland Asia, Pacific Oceania, the Middle East, Europe, the Caribbean, and throughout North America. He is broadly published in the technical literature, and co-author of books on urban ecology and the ecology and toxicology of true viper snakes. He provides weekly insights regarding environmental sustainability in a column published on the AEHS Foundation web site, titled "PeopleSystems and Sustainability: This Week in the Global Environment".

POTENTIAL OF SAFFLOWER (*CARTHAMUS TINCTORIUS L.*) FOR PHYTOREMEDIATION OF SOILS CONTAMINATED WITH HEAVY METALS

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ABSTRACT

A field study was conducted to evaluate the efficacy of using safflower plants for phytoremediation of contaminated soils in the absence and presence of organic soil amendments (compost and vermicompost). The experiment was performed on an agricultural field contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The field experimental was a randomized complete block design containing five treatments and four replications (20 plots). The treatments consisted of a control (no organic amendments), compost amendments (added at 20 t/daa and 40 t/daa), and vemicompost amendments (added at 20 t/daa and 40 t/daa). Heavy metal contents in roots, stems, leaves and seeds of safflower were analyzed. Safflower can be referred to as cadmium hyper accumulators, as well as accumulators of lead and zinc and can be successfully used in phytoremediation of heavy metal polluted soils. Tested organic amendments significantly influenced the uptake of Pb, Zn and Cd by safflower plant. The potential of safflower to uptake and accumulate cadmium, lead and zinc in leaves increased after the 40 t/ha vermicompost treatments. The compost and vermicompost treatments significantly reduced heavy metals concentration in safflower seeds, but the effect differed among them. The possibility of further industrial processing of seeds to oil and using the obtained oil will make safflower economically interesting crops for farmers of phytoremediation technology.

Keywords: heavy metals, safflower, phytoremediation, organic amendments

1. INTRODUCTION

Heavy metal contamination of agricultural soils is a worldwide problem. The remediation of metal contaminated sites often involves expensive and environmentally invasive civil engineering-based practices. A range of technologies such as fixation, leaching, soil excavation, and landfill of the top contaminated soil ex situ have been used for the removal of metals. Many of these methods have high maintenance costs and may cause secondary pollution or have an adverse effect on biological activities, soil structure, and fertility (Marques et al., 2008). Phytoremediation is an emerging technology, which should be considered for remediation of contaminated sites because of its cost effectiveness, aesthetic advantages and long term applicability. This technology can be defined as the efficient use of plants to remove, detoxify or immobilize environmental contaminants in soils, waters or sediments through the natural,

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biological, chemical or physical activities and processes of the plants. It is best applied at sites with shallow contamination of organic, nutrient or metal pollutants (Wang et al., 2005). This plant based technique is essentially an agronomic approach and its success depends ultimately on agronomic practices applied at the site. The addition of organic matter amendments, such as compost, fertilizers and wastes, is a common practice for immobilization of heavy metals and soil amelioration of contaminated soils (Clemente et al., 2005). Organic amendments are able to improve the physical, chemical and biological properties of soil by: (i) raising the pH, (ii) increasing the organic matter content, (iii) adding essential nutrients for plant growth, (iv) increasing the water holding capacity, and (v) modifying heavy metals bioavailability (Walker et al., 2003, 2004).

Some research has shown that the amendment of contaminated soils with organic matter reduced bioavailability of heavy metals (Khan et al., 2000). Organic soil matter has been of particular interest in studies of heavy metal sorption by soils, because of the tendency of transition metal cations to form stable complexes with organic ligands. Organic matter is known to form strong complexes with heavy metals (Krogstad, 1983). The content of organic matter affects speciation of heavy metals in soil (Lo et al., 1992). High organic matter content was reported to decrease concentrations of Cd and Ni in the soil solution (Arnesen and Singh, 1999). This is very important because a high content of organic matter in contaminated soil is one of the ways to exclude heavy metals from the trophic chain. Cow manure, poultry manure and pig manure were found to be effective in reducing lead availability to plants, leading to lower uptake of lead (Ye et al., 1999). The use of composts has been recognized generally as an effective means for improving soil aggregation, structure and fertility, increasing microbial diversity and populations, improving the moisture-holding capacity of soils, increasing the soil cation exchange capacity (CEC) and increasing crop yields (Zink and Allen, 1998).

Vermicompost contains the most nutrients in plant-available forms such as nitrates, phosphates, and exchangeable calcium and soluble potassium (Orozco et al., 1996). There is accumulating scientific evidence that vermicomposts can influence the growth and productivity of plants significantly. Various greenhouse and field studies have examined the effects of a variety of vermicomposts on a wide range of crops including cereals and legumes (Chan and Griffiths, 1988), vegetable, ornamental and flowering plants (Atiyeh et al., 2000), and field crops (Arancon et al., 2006).

The use of crop plants for phytoremediation of contaminated soils has the advantages of high biomass production and adaptive capacity to variable environments (Komarek et al., 2007; Fassler et al., 2010). However, to succeed they must be tolerant to the contaminants and be capable of accumulating significant concentrations of heavy metals in their tissues. Additionally, crops could make the long time periods for decontamination more acceptable, economically and environmentally. [If the contaminated biomass may be further proceed for added value products (not only concentrated on deposits of hazardous wastes), then such fact represents an improvement of economical efficiency of phytoremediation technology]. Industrial plants i.e., energy crops or crops for bio-diesel production are therefore the prime candidates as plants for phytoremediation. The use of energy and/or bio-diesel crops as plants for phytoremediation would give contaminated soil a productive value and decrease remediation costs.

The main objective of this paper is to conduct a systematic study, which will help determine the impact of organic soil amendments on the uptake of heavy metals by safflower (*Carthamus*

Tinctorius L.), as well as the possibilities to use the plant for phytoremediation of heavy metal contaminated soils.

2. MATERIALS AND PROCEDURE

The experiment was performed on an agricultural field contaminated by the Non-Ferrous-Metal Works near Plovdiv, Bulgaria. The field experimental was a randomized complete block design containing five treatments and four replications (20 plots): 1 - introduction of 20 t/daa of vermicompost to the soil, 2 - introduction of 40 t/daa of vermicompost to the soil, 3 - introduction of 20 t/daa of compost to the soil, 4 - introduction of 40 t/daa of compost to the soil, 5 - control variant.

Characteristics of soils and organic amendments are shown in Table 1. The soils used in this experiment were slightly acidic, with moderate content of organic matter and essential nutrients (N, P and K) (Table 1). The psedo-total content of Zn, Pb and Cd is high (1430.7 mg/kg Zn, 876.5 mg/kg Pb and 31.4 mg/kg Cd) and exceeds the maximum permissible concentrations (320 mg/kg Zn, 100 mg/kg Pb, and 2.0 mg/kg Cd).

To determine the effect of the organic amendments, the soil samples were collected 1 month after addition of organic amendments. A soil subsample was air-dried passed through a 2-mm sieve and characterized for soil pH (H_2O) in deionised water suspension of 1:5 (w/v); total nitrogen by the Kjeldahl method (N Kjeldahl) total oxidizable organic carbon according to Tube digestion method (with titration) (Sparks, 1996).

The pseudo-total and DTPA-extractable concentration of heavy metals, micro and macroelements in the soils, after four weeks' equilibration, were determined. Pseudo-total content of metals in soils was determined in accordance with ISO 11466. The available (mobile) heavy metals contents were extracted by a solution of DTPA (1 M NH_4HCO_3 and 0.005 M DTPA, pH 7.8). The same procedures were applied to organic amendments.

Table 1. Characterization of the soil and the organic amendments used in the experiment

Parameter	Soil	Compost	Vermicompost
pH	6.5	6.9	7.5
EC, dS/m	0.2	0.2	2.2
Organic matter,%	3.86	72.1	38.58
N Kjeldal,%	0.236	2.223	1.569
C/N	9.41	32.43	24.59
Pseudo-total P, mg/kg	642	12653.9	10210.8
Pseudo-total K, mg/kg	5517.5	6081.7	10495.1
Pseudo-total Pb, mg/kg	876.5	12.02	32.25
Pseudo-total Zn, mg/kg	1430.7	170.77	270.3
Pseudo-total Cd, mg/kg	31.4	0.192	0.686

The test plant was safflower (*Carthamus tinctorius L.*). Safflower (*Carthamus tinctorius* L., Asteraceae) is an annual oilseed crop that has been cultivated on small plots in the world. Safflower is one of the alternative oil crops, particularly in dry land due to tolerance to cold,

drought and salinity stress (Weiss, 2000). Safflower seeds were sown in each plot; between row and within row distances were 60 and 20 cm, respectively. Each hole was 5–6 cm deep, containing 3 seeds. After safflower had grown for 15 days, the safflower was thinned to one plant per hole.

Upon reaching commercial ripeness, the safflower plants were gathered and the concentrations of Pb, Zn and Cd in their different parts (roots, stems, leaves and seeds) were determined by the method of dry mineralization.

Statistical analyses were conducted with Statistica v. 7.0. Pearson's linear correlations were used to assess the relationships among pH, the soil organic content and available Pb, Zn and Cd in soil.

3. DATA AND ANALYSIS

3.1 Assess the Impact of Amendments on the Main Soil Parameters

The effects of compost and vermicompost on pH, EC, soil organic content and total N are summarized in Figure 1. The results obtained showed that the soil organic properties depended on the type and rate of the soil amendments and treatment.

3.1.1 pH

Soil pH varied with amendment treatment (Fig.1). Application of compost reduced the soil pH significantly as compared to control, whereas application of vermicompost increased soil pH. The direction of the change in soil pH as a result of treatment application reflected the initial pH of the amendment material.

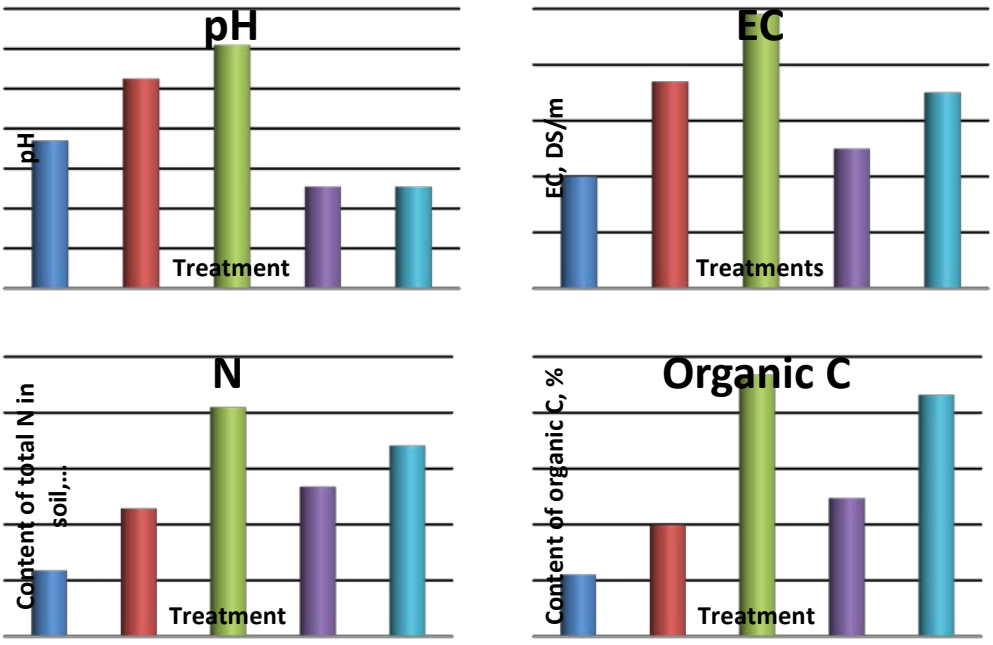

Figure 1. Influence on compost and vermicompost on pH, EC, soil organic C and total N

The increase in pH could be due to the higher pH value of the vermicompost (pH of 7.5) in relation to soil (pH of 6.5). However, this increase is not considered dangerous to soil quality because the values remained close to neutrality. The sensitivity of soil pH to the organic amendments was likely due in part to the low buffering capacity (Neilsen et al., 1998). The addition of vermicompost increased soil pH and pH increased with the higher dose of vermicompost. Contrary, Atiyeh et al. (2001) reported that an increase of the vermicompost rate in soil resulted in the decrease in soil pH. The production of NH_4^+, CO_2 and organic acids during microbial metabolism in vermicompost may be contributed to the decrease in soil pH (Albanell et al., 1988).

The addition of compost decreased soil pH. The obtained results confirm findings from Walker et al. (2003) that an addition of compost to soil led to a decrease in soil pH. Smiciklas et al. (2002) also observed a decrease in soil pH after the use of organic materials. The production of organic acids (amino acid, glycine, cystein and humic acid) during mineralization (amminization and ammonification) of organic materials by heterotrophs and nitrification by autotrophs would have caused this decrease in soil pH.

3.1.2 Organic Carbon

Organic matter plays an important role in soil, because of its higher CEC and water holding capacities, as well as its chelation ability and influence on soil stability. It is considered to be a good resource of available elements. It improves soil structure, aeration and aggregation (Sparks, 1995).

The increase of soil organic carbon with addition of organic amendments to soil is caused by the high organic matter content of compost and vermicompost (Table l). The application of organic amendments led to a significant increase in organic matter content compared to its initial level (Fig. 1). The soils treated with compost and vermicompost exhibited a higher organic content than the control. Therefore, the addition of compost was able to affect the soil organic matter content, however, in contrast to previous data (Giusquiani et al., 1995), our data demonstrates that the organic content increase was proportional to the compost dose used.

The organic content reached from 2.2% in the control to 3.99 and 9.36% as a result of adding vermicompost at rate of 20 t/daa and 40 t/daa, respectively. Compost application led to an increase of organic content to 4.95 and 8.64%. Both tested organic amendments had the capacity to raise soil organic matter (Fig. 1), and there is no significant difference between these amendments in spite of the higher large organic matter content in the compost (72.9%). The use of organic amendments increases the soil organic carbon and improves soil structure. Fortuna et al. (2003) argued that the vermicompost amendment could increase the carbon content up to 45% of the original levels, and thus contribute to increase the soil structural stability, particularly that of the macroaggregates.

3.1.3 Total N

The changes in the organic content in soils brought about changes in the total nitrogen content (Fig.1). There was a strong positive correlation between the total organic content and the total nitrogen content.

The results showed that the total N concentration in soil was significantly affected by compost and vermicompost treatments. The soils treated with vermicompost at the rate of 40t/daa had more total N compared to soils without vermicompost application.Vermicompost might have produced more residual N in soil than those in control plots.There have been other reports of an increase of N in soil after application of vermicompost (Nethra et al., 1999).

3.1.4 Soil C/N Ratio

The soil C/N ratio is often used to explain different turnover rates for early residue decomposition.The C/N ratios of the soils are narrow. N can be easily mineralized when the C/N ratio is less than 20:1. According to Mikkelsen and Hartz (2008), the C/N ratio of added organic materials is a good, but not an absolute, predictor of whether N immobilization is likely (C/N ratio > 25:1) or if mineralization is likely (C/N ratio < 20:1). In our study, the mean of C/N of soil was narrow (9.41 -12.67), below 20 in all soils.

3.2 Mobile Content of Heavy Metals (Pb, Cd, Zn)

Fig. 2 represents the results for quantities of mobile forms of DTPA extracted Pb, Cd and Zn from naturally contaminated soil from the region of Plovdiv and their change after adding organic soil amendments. An important factor influencing the mobility of Pb, Cd and Zn is the quantity of organic matter in the soil (McGrath et al., 2000). Many authors have found that soils with high organic carbon content, as well as adding organic fertilizer, the cadmium content in soil decreases. This effect is explained by the high cation exchange capacity of organic matter and its ability to form chelate complexes with Cd. Haghiri (1974) found that reducing the cadmium content of plants by increasing the amount of imported organic ameliorants due to higher cation exchange capacity of the soil.

Organic amendments affect DTPA-extractable heavy metals. The results show that organic supplements affect the amount of mobile zinc differently. Quantities extracted with DTPA mobile forms of zinc increases with the amount rate of the compost to soils. Application of vermicompost reduces the amount of available Zn to 193.1 mg/kg, while the addition of compost led to its increase to 328. 8 mg/kg. The total Zn content, pH, organic matter, adsorption sites and microbial activity of the soil affect the Zn availability (Alloway, 1995). The soil pH is the most important factor controlling Zn availability, which decreases with the increase of the pH (Shuman, 1999). In this experiment the increase of Zn availability after compost application was attributed to pH reduction and greater organic matter degradation. Shuman (1999) found that retention of Zn in the soil increased in the presence of organic fertilizers. Zn can form insoluble compounds - precipitates during the mineralization of organic meliorants (Walker et al., 2003) and insoluble compounds in the form of $ZnCO_3$ in calcareous soils (Usman et al., 2004). Shuman (1999) found that pH, clay content, organic matter and cation-exchange capacity influence the adsorption of Zn in soils. Mandal and Hazra (1997) found that the addition of organic amendments and lower soil pH leads to an increase in the amount of available Zn. A

correlation between the content of mobile zinc and soil pH and between mobile zinc and organic matter in the soil was found.

Similar are the results obtained with respect to cadmium. Application of vermicompost reduced the amount of DTPA-extractable mobile forms of Cd from the soil, while compost can even increase Cd to 18.8 -19.2 mg/kg. Similar results were obtained from Karaca (2004) on the application of mushroom compost and grape marc. Reducing extracted with DTPA mobile forms of Cd may be due to the high cation exchange capacity of organic matter and the ability to connect Cd from the soil. Korcak and Fanning (1985) found a positive correlation between DTPA-extractable mobile forms of Cd and quantity organic matter content in the soil.

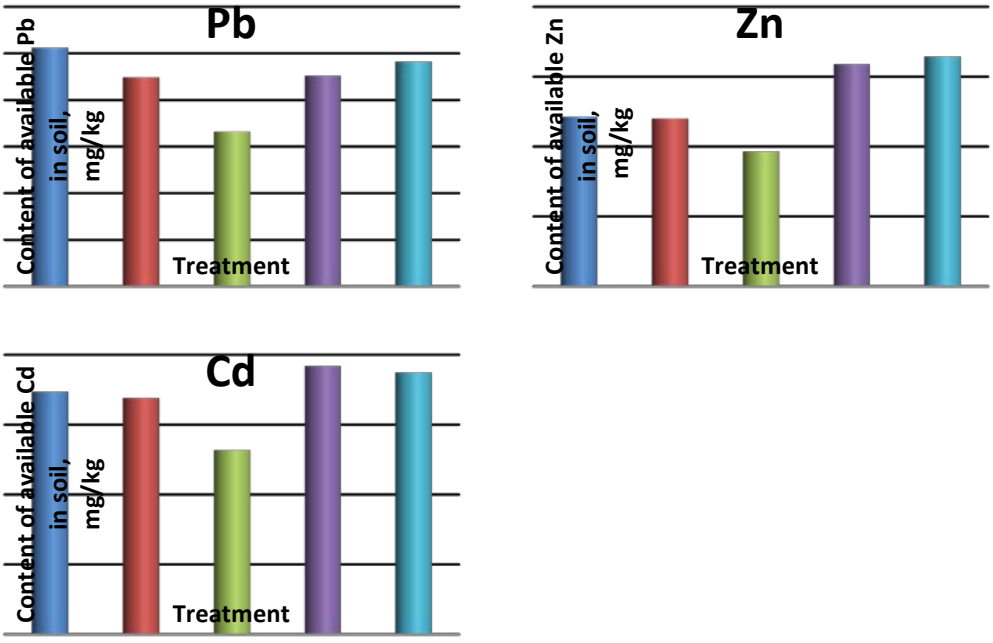

*Figure 2***.** Effect of different organic amendments (compost and vermicompost) applications to soils on availability of heavy metals

A downward trend in mobile forms by the action of soil amendments is expressed more clearly in terms of Pb. In most cases, mobile forms of Pb diminished.

Humic acids from organic amendments tend to form complexes that are different for each metal, and also depend on soil conditions such as pH, cation exchange capacity and clay mineral fraction (Barancikova and Macovnikova, 2003). Organic matter not only forms complexes with these metals, but it also retains them in exchangeable forms, affecting each metal differently. Some metals are bound and rendered unavailable while others are bound and readily available (Kononova, 1966). The results of the present study indicated that enrichment of soil with organic matter could reduce the content of bioavailable metal species as a result of complexation of free ions of heavy metals. This is indicative of heavy metals immobilization by humic substances from compost and vermicompost application. Results appear to verify the function of humic acid in improving phytoremediation efficiency of soils contaminated with heavy metals, and that the potential environmental availability of metals may be controlled by soil organic amendments.

3.3 Uptake of Heavy Metals from Safflower (Control)

To clarify the issues of absorption, accumulation and distribution of heavy metals in vegetative and reproductive organs of safflower were analyzed using samples of roots, stems, leaves and seeds.Table 2 presents the results obtained for the content of heavy metals in the vegetative and reproductive organs of the study oilseed crop.

*Table 2***.** Content of heavy metals (mg/kg) in safflower

Element	Roots	Stems	Leaves	Seeds
Pb	142.8±2.5	86.3±0.8	580.5±3.6	3.6±0.2
Cd	52.4±1.3	29.4±0.5	148.2±2.1	2.9±0.1
Zn	436.0±5.3	207.5±2.8	651.9±6.8	133.5±2.0

nd – not detectable

3.3.1 Content of Heavy Metals in Root System

Considerably lower values were established in the roots of safflower compared to the aboveground parts of safflower. The content of Pb in the roots of safflower without amendments reached to 142.8 mg/kg, Zn – 436.0 mg/kg and Cd -52.4 mg/kg. The obtained values for the heavy metals (Cd, Pb and Zn) in the roots are much higher than the values considered of Liphadzi and Kirkham (2005) as toxic to plants (0.1 mg/kg Cd, 30 mg/kg Pb, 100 mg/kg Zn).

Sayyad et al. (2010), Shi et al. (2010) and Namdjoyan et al. (2011) studies lead to the conclusion that the safflower plant has a relatively high potential for accumulation of Cd in its roots. According to Pourghasemian et al. (2013), safflower has a higher tolerance to Cd due to the high capacity of its roots to absorb and accumulate it. The high concentrations in the roots may be due to the efficient bonding and fragmentation to the vacuoles of glutathione and phytochelatins (Molina et al., 2008).

The fact that the exposure to Cd increases the content of phytochelatins in the roots shows the potential role of phytochelatins in accumulating this element and binding to the roots of the safflower plant. According to Namjooyan et al. (2012), safflower has a great ability to adapt to the toxicity of Cd. The accumulation of Cd is significantly affected by its content in the soil solution, and the rapid growth and easy harvesting of the safflower plant are important qualities in the use of this plant in research related to phytoremediation.

There are no published data in the scientific literature on the content of heavy metals in safflower grown under field conditions, which makes it difficult to conduct a comparative analysis between our results and the results of other authors. Perhaps this is the reason for the non-conformances between the obtained results and those from potted experiments done by other authors.

According to Namdjoyan et al. (2011) and Namjooyan et al. (2012) and Pourghasemian et al. (2013), heavy metals mainly accumulate in the root system of the safflower plant and significant content in the roots can cause damage to the root system, something which was not observed in our experiment. The roots are the first organ exposed to the toxicity

of metals and therefore they are the affected organ in the studied safflower varieties (Namdjoyan et al., 2011, Namjooyan et al., 2012).

The reason for this is that when the plants are grown under field conditions, the roots penetrate deeply into the soil and easily avoid areas with contaminated soil, whereas in potted experiments the plant roots are in contact only with the contaminated soil, which is probably the reason why the safflower roots accumulate significantly greater amounts of heavy metals. The safflower plant is characterized by a well-developed root system with a taproot that penetrates to a depth of 2-3 m and side branches reaching 60-80 cm sideways and 20-30 cm in depth.

This is probably the reason why it accumulates a smaller amount of heavy metals in the roots, grows normally and produces seeds in contaminated soils when grown under field conditions.

3.3.2 Content of Heavy Metals in Aboveground Parts

A significant accumulation of lead has been detected in the leaves and stems of the safflower plant. The content of this element reaches amounts of 86.3 mg/kg in the stems and 580.5 mg/kg in the leaves, and is much higher than the toxic levels for animals - 30 mg/kg (Chaney, 1989). The total content of Pb in the soil from the field experiment reached up to 876.5 mg/kg and this substantially exceeds the default values of 100 mg/kg for toxic effects on plants (Kabata-Pendias, 2001).

Numerous studies show that only a small fraction of the available Pb in the soil is absorbed by most plants. It is also known that most of the absorbed Pb is accumulated in the roots and it does not move to the aboveground parts of the plants. Thus, its entering the food chain can be prevented by the "soil-plant barrier"(Chaney, 1989), thereby preventing contamination of the leaves and seeds, which are used for animal forage.

Our results, however, show a significant ability of the safflower plant to accumulate lead in its leaves. This is probably due to the anatomical and morphological characteristics of this culture. The greater accumulation in the leaves is probably due to the fact that they are leathery and covered with thorns (i.e., they have a xerophytic character), which determines their drought tolerance and lesser evaporation of water and contained salts.

The content of Cd in the stems and leaves of the safflower plant reaches 29.4 mg/kg and 148.1 mg/kg, values considered to be toxic to plants. According to Kabata-Pendias (2001), a value of 5.0 mg/kg is considered to be toxic to plants. Our results demonstrate the unique ability of safflower to accumulate Cd in its aboveground biomass. The content of Zn in the safflower stems and leaves reached values of 207.5 mg/kg and 651.9 mg/kg, and these values are higher than the critical values for plants(100-400 mg/kg).

According to Sayyad et al. (2009), the safflower plant accumulates a moderate amount of heavy metals in its aboveground biomass. Our results do not confirm those found by Sayyad et al. (2009), Namdjoyan et al. (2011) and Namjooyan et al. (2012) that the content of cadmium is lower in the aboveground biomass compared to the roots, and Cd partially moves from the roots to the aboveground biomass. Symptoms of Cd toxicity are not observed in spite of the high concentrations of cadmium in the leaves. It’s probable the safflower plant can hyperaccumulate cadmium to the vacuoles in the cells of the

leaves and thus avoid Cd toxicity at the cellular level (Ozturk et al., 2003; Uraguchi et al., 2006). This phenomenon can be seen as a consequence of strong pressure at high concentrations of heavy metals in the soil. Therefore, safflower can be grown in contaminated soils and can be used as an appropriate culture in phytoremediation of cadmium contaminated soils.

The physiological mechanisms related to the tolerance of plants to Cd toxicity have not yet been well studied. The differences in the absorption of Cd by the roots and its accumulation in the aboveground biomass can be explained with the genotypic variations in the tolerance to Cd toxicity (Ozturk et al., 2003). By increasing the duration of exposure, there is a significant reduction of the aboveground biomass, especially at higher Cd concentrations in the soil solution. According to Namdjoyan et al. (2011) and Namjooyan et al. (2012), the toxic effect of Cd is manifested in reduced plant biomass, which is reduced at the highest concentrations of Cd and the most protracted exposures.

3.3.3 Content of Heavy Metals in Seeds

The heavy metal content in the seeds is significantly lower compared to the root system and the aboveground biomass of the plants. Their accumulation into the safflower seeds is likely to occur through the vascular tissue system of the plant. The content of Pb and Zn in the seeds of the control is 3.57 mg/kg and 133.5 mg/kg and does not reach the critical value of 30 mg/kg for Pb and 300 mg/kg for Zn (Hapke, 1991). Cd accumulates in the seeds (2.9 mg/kg) at levels significantly above the recommended maximum levels tolerated by animals (0.5 mg Cd /kg, Chaney, 1989) and the recommended values for food (1 mg/kg) (Hapke, 1991).

3.4 Influence of Organic Amendments on the Accumulation of Heavy Metals in the Vegetative and Reproductive Organs of Safflower

Introduction of compost and vermicompost significantly affect the uptake of heavy metals (Pb, Zn and Cd) of the safflower plants. Adding compost lowers the content of Pb, Zn and Cd in the roots of the safflower plant and this decrease is more pronounced with the introduction of 20 t/daa compost. The content of Pb in the roots decreases to 99.8 mg/kg, and that of Cd to 11.7 mg/kg (Fig. 3). Adding compost also leads to lower contents of Pb, Zn and Cd in the stems of the safflower plant, and this decrease is more pronounced for lead and zinc, and in the case of cadmium - with the introduction of 20 t/daa of compost. The introduction of 20 t/daa of compost leads to a slight decrease in the content of heavy metals in the leaves of the safflower plant, while in the case of introduction of 40 t/daa of compost, there is a slight increase in the levels of lead, zinc and cadmium.

These results can be explained with the amount of mobile forms of heavy metals. The addition of 20 t/daa of compost to the soil leads to a decrease of the mobile forms of lead, zinc and cadmium, resulting in a significantly lower content of elements found in the leaves of the plants. The introduction of 40 t/daa of compost increases the mobile forms of zinc and cadmium, which corresponds to the higher content of these elements in the leaves of the safflower plant.

Adding vermicompost also lowers the content of heavy metals in the roots and stems compared to the control, and this decrease is more pronounced with the introduction of 40 t/ daa of

vermicompost. The Pb content decreases from 142.8 mg/kg to 63.9 mg/kg, the Zn content from 436 mg/kg to 67.5 mg/kg, and that of Cd - from 52.4 mg/kg to 6.0 mg/kg.

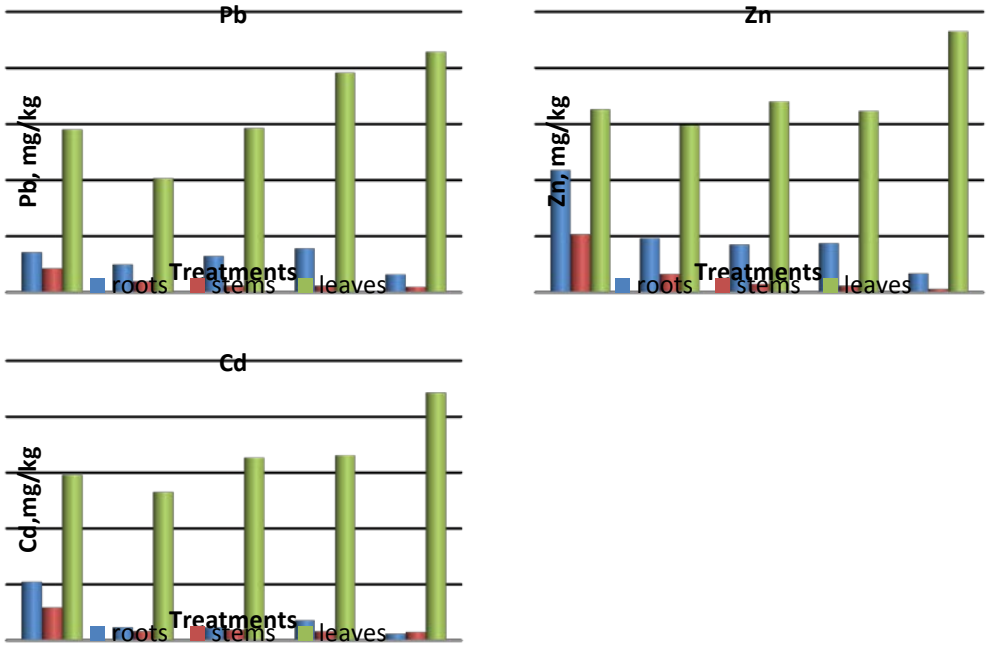

Figure 3. Effect of different organic amendments (compost and vermicompost) applications to accumulation of heavy metals in vegetative organs of safflower

Similar reduction tendency is observed with the amount of lead, zinc and cadmium in the stems, wherein the lead content decreases from 86.3 mg/kg to 19.9 mg/kg, the content of zinc from 207.5 mg/kg to 11.96 mg/kg, and that of cadmium from 29.4 to 7.4. In regards to the leaves, the content of heavy metals tends to increase. A relationship has been found between their content in the safflower leaves and the quantity of imported vermicompost. By increasing the amount of vermicompost, the content of Pb in the leaves increased from 580.5 mg/kg in the control to 857.8 mg/kg after the introduction of 40 t/daa of compost, the content of Zn from 651.9 mg/kg, to 931.1 mg/kg and the content of Cd from 148.1 mg/kg to 221.5 mg/kg.

Introduction of compost leads to a lower content of heavy metals, and as with most elements, with the increase of the amount of additive, the decrease is greater. With the introduction of vermicompost, the tendency is the same. The influence of organic ameliorants on the accumulation of heavy metals in the safflower seeds depends essentially on their quantity.

The reduction of the heavy metal content in the seeds compared to the control is strongly expressed, with the compost variant, the Pb content decreases from 3.6 mg/kg to 1.4 mg/kg, while in the vermicompost variant there was a decrease to 1.5 mg/kg (Fig.4) and these values are lower than the maximum feed concentration (30 mg/kg). Relationship has been found between the amount of the imported ameliorant and the Pb content in the safflower seeds. With the increasing amount of the ameliorant, the Pb content decreases. The results for cadmium are similar. The reduction of the Cd content in the seeds compared to the control is strongly expressed, as in the variants with the introduction of 40 t/daa of compost and 40 t/daa of

vermicompost, the Cd content in the seeds decreases from 2.9 mg/kg to 0.5 mg/kg and these values are within the limits for forage (Hapke, 1991).

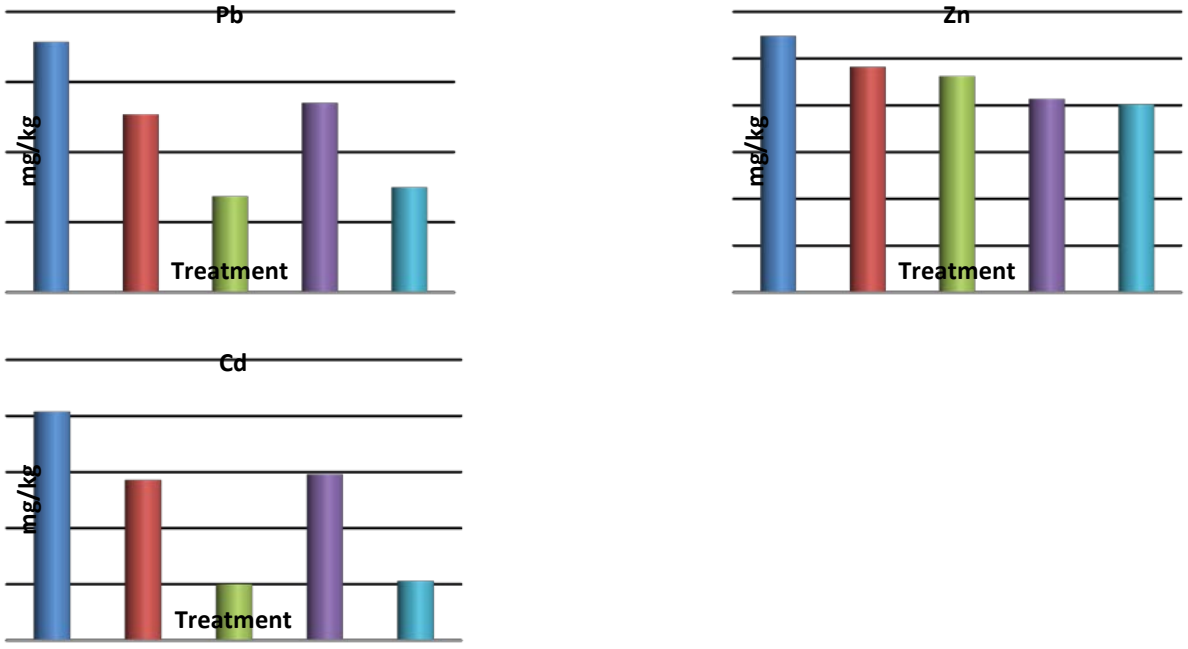

Figure 4. Effect of different organic amendments (compost and vermicompost) applications to accumulation of heavy metals in reproductive organs of safflower

The content of Zn in the safflower seeds after the introduction of organic ameliorants reduces. This decrease is more pronounced after the introduction of vermicompost (from 109.6 mg/kg to 84.5 mg/kg), while with the introduction of compost, the decrease is significantly less - to 92.4 mg/kg. No correlation has been established between the amount of the imported organic ameliorants and the content of Zn in the safflower seeds. However, the introduction of organic additives lowers the amount of zinc to levels that are lower than the maximum levels for forage.

3.5 Uptake of Heavy Metals from Safflower

The mobility of heavy metals from the soil into the roots of the plants and the ability of the plants to transport metals from their roots to their aboveground biomass are estimated by calculating the bioconcentration factor (BCF) and the translocation factor (TF). BCF is defined as the ratio of the metal content in the root biomass to the metal content in the soil, and TF is the ratio of the metal concentration in the aboveground biomass to that in the root biomass (Yoon et al., 2006).

Tables 3 and 4 show the results for the BCF and the TF, which were calculated for the safflower from the experimental control and the variants after the introduction of organic ameliorants. The BCF values for lead are < 1, which indicates that the concentration of this element does not exceed its content in the soil. For cadmium and zinc, these values are

greater than 1. There are significant differences between the values obtained from the control and the variants treated with organic ameliorants variants. Introduction of ameliorants leads to a significant decrease in the BCF values. The BCF values can be used as an indicator for assessing the ability of the plants to accumulate heavy metals depending on the content of metals in the soil. These values vary depending on the concentration and type of heavy metals, the ability of the plants to accumulate them, the physiological characteristics of the plants and the environmental factors.

Mattina et al. (2003) is doing research into the BCF values in lettuce, cucumbers and other vegetables grown in soils contaminated with chlorides and heavy metals. Their results suggest that these plants have different BCF values when exposed to the action of various heavy metals. Similar results have been obtained by Zhi-xin (2007) for sunflower, according to which the influence the BCF has is not only on the content of Cd and Pb, but also the state of the crop. The TF values show the level of movement of the metal absorbed by the root system of the plant to its aboveground biomass. Generally, the TF doesn't show the same tendency as the one observed with the BCF. The introduction of compost and vermicompost leads to an increased translocation factor, which shows the enhanced ability of the plants to bioaccumulate heavy metals as compared with the control.

Table 3. Uptake of heavy metals by safflower (Bioconcentration factor (BCF))

Element	Control	Compost		Vermicompost	
		20 t/daa	40 t/daa	20 t/daa	40 t/daa
Pb	0,28	0,22	0,27	0,35	0,19
Cd	3,02	0,61	0,63	1,06	0,46
Zn	9,61	0,60	0,52	0,73	0,35

$BCF=[Metal]_{roots}/[Available\ Metal]_{soil}$

After the introduction of vermicompost, the TF values for cadmium increased from 3 to 38 and this indicates that a very large proportion of Cd moves to the aboveground parts of the safflower. Introduction of compost also leads to an increase of the TF, but this increase is significantly less (up to 14.6). In terms of Pb, after the introduction of 40 t/daa of vemicompost, the TF values increased from 4.6 to 13.7. Obviously, the introduction of 40 t/daa of vermicompost leads to an increased capacity of the plant to bioaccumulate Pb in comparison with the control and the other variants. Similar results were obtained for zinc. After the introduction of compost, the TF values for zinc increased from 1.97 to 4.15 and after the introduction of 40 t/daa of vermicompost - up to 13.97. In the variant with 40 t/daa of vermicompost, the TF values show that a significantly greater amount of zinc moves to the leaves in comparison with the other variants.

*Table 4***.** Uptake of heavy metals by safflower (Translocation factor (TF))

Element	Control	Compost		Vermicompost	
		20 t/daa	40 t/daa	20 t/daa	40 t/daa
Pb	4,67	4,48	4,71	5,13	13,73
Cd	3,39	12,12	14,59	9,69	38,06
Zn	1,97	3,42	4,15	3,84	13,97

$TF= [Metal]_{above\ ground\ parts}/[Metal]_{roots}$

According to Pourghasemian et al. (2013), a correlation between the ratio of the aboveground biomass to the roots and the temperature, or a correlation between the absorption of cadmium by the leaves and the temperature haven't been established. Cd shows no significant influence on the aboveground biomass/root biomass ratio in the safflower genotypes, and according to the authors, it can be assumed that Cd does not affect the distribution of assimilants between the roots and the aboveground biomass. It was found that the movement of Cd can occur via both the apoplastic and symplastic pathways, as is the case with S. alfredii (Lu et al., 2009). It was also found that low temperature can slow down the movement of Cd via the symplastic pathway. In this way, the movement of Cd from the roots to the stems of the safflower species, where the symplast plays the dominant role in the transport of metals, may increase significantly compared to the other species. Further research is needed in order to make sure whether the transport of Cd in the safflower plant primarily occurs via the symplastic or the apoplastic pathway, but the greater movement at a higher temperature shows that the symplastic pathway dominates in this oilseed crop. According to Pourghasemian et al., (2013), the rising of temperature causes greater movement of Cd and greater sensitivity to Cd. According to Shi et al. (2010), the safflower plant is less tolerant to high concentrations of heavy metals and this species can be considered to be a potential accumulator of Cd.

3.6 Safflower – Accumulator or Hyperaccumulator?

To determine the extent to which safflower accumulates heavy metals and to which group of plants it can be assigned (hyperaccumulators or accumulators), we used the existing criteria for determination of heavy metal accumulation in plants. Plants have the ability to accumulate metals and some of them can accumulate them in large quantities (100 times more than other plants in the same conditions, without showing any adverse effects). Plants that have the ability to tolerate and accumulate heavy metals in quantities greater than the toxic levels are known as hyperaccumulators. In recent years, the number of studies on the use of hyperaccumulators for remediation of contaminated areas, owing to the capacity of these plants to absorb heavy metals from contaminated soil and accumulate them in their aboveground biomass, has been steadily increasing.

The classification of plants as hyperaccumulators, accumulators, indicators and non-accumulator species is based on precise and clear criteria (Mganga et al., 2011). Plants that contain high quantities of heavy metals in their root biomass, but whose aboveground biomass/root biomass ratio is less than 1, belong to the non-accumulator species. According to Baker and Walker (1990), indicator species are those whose heavy metal content is proportionate to the heavy metal content in the soil. Plants can be classified as hyperaccumulators if they meet the following criteria: (a) have an aboveground biomass/root biomass ratio > 1; (b) have an extraction ratio (the ratio of the heavy metal content in the aboveground biomass to the heavy metal content in the soil) > 1; (c) have a heavy metal content which is 10-500 times higher than that of plants grown in noncontaminated soils; and (d) contain more than 1000 mg/kg of copper, lead, nickel, chromium, more than 100 mg/kg of cadmium, or more than 10,000 mg/kg of zinc (McGrath and Zhao 2003).

To be able to give a definite answer to the question of what the capacity of the safflower plant to extract heavy metals from the soil is, we will examine the above criteria one by one.

3.6.1 Opportunity for Accumulation of Heavy Metal from Safflower

Our results show that safflower has the ability to accumulate cadmium, lead and zinc in its aboveground biomass and can be assigned to the hyperaccumulators of cadmium, as the Cd content in its aboveground biomass exceeds 100 mg Cd / kg. The aboveground biomass of the safflower from the control accumulated Pb up to 580 mg/kg, and Zn up to 651.9 mg/kg, but these values do not reach 1000 mg/kg for Pb and 10,000 mg/kg for Zn, and so the plant can be assigned to the accumulators of lead and zinc.

3.6.2 Tolerance to Metals

Safflower is a crop which is tolerant to heavy metals and which can be grown in soils contaminated with heavy metals. The safflower plants are characterized by a great capacity to absorb and accumulate cadmium and lead, but show no signs of toxicity (chlorosis and necrosis) at levels of 31.4 mg/kg of Cd content and 876.5 mg/kg of Pb content in the soil. The biomass of the plants is an indirect indicator of their tolerance to toxic metals. The experiment which we conducted showed that the safflower from the experimental control has a sufficient biomass and can be used for phytoextraction of Cd from the contaminated soil *in situ*. The introduction of 40 t/daa of vermicompost leads to an increase of the biomass (the number of branches and inflorescences) and also to an increased potential of the safflower plant to absorb cadmium, lead and zinc (10, 3 and 6 times more, respectively).

3.6.3 Translocation Factor (TF)

This specific criterion for hyperaccumulators can reach values > 1, which indicates that the content of heavy metals in the aboveground biomass is higher than that in the underground parts (the root biomass). Therefore, this criterion is extremely important in phytoextraction where harvesting of the aboveground biomass of the plants is the main objective (Karami and Shamsuddin, 2010). According to McGrath and Zhao (2003) regarding hyperaccumulators, both the BCF and the TF indices must be > 1. According to Sarma (2011), more than 500 plant species from 101 families have been classified as hyperaccumulators. According to McGrath and Zhao (2003), hyperaccumulation of Cd and As is rare in plant species. They have found that basically hyperaccumulators have a small biomass with a long vegetation period and only a few plants with a large biomass have the ability to accumulate heavy metals. Baker et al. (2000), however, identifies many species that can be classified as hyperaccumulators on the basis of their ability to tolerate toxic concentrations of metals such as Cd, Cu, As, Co, Mn, Zn, Ni, Pb and Se.

3.6.4 Bioaccumulation Factor (BAC (EF))

The effectiveness of phytoextraction is also determined by the bioaccumulation factor or enrichment factor (McGrath and Zhao, 2003). The bioaccumulation factor is defined as the ratio of the concentration of the metal in the aboveground biomass of the plant to its concentration in the soil, and is a measure of the ability of the plant to absorb and transport metals to its aboveground biomass, which can be easily harvested. In hyperaccumulators, the enrichment factor is greater than 1 and in some cases may reach values of 50-100.

Our results show that the value of the bioaccumulation factor for the control plants reaches 1.3 for lead, 3.5 for zinc and 10 for cadmium (Table 5). Therefore, safflower can be used for phytoextraction because the number of plants required to reduce the concentration of the metal in the topsoil by half is less than 100. The introduction of 40 t/daa of vermicompost leads to a significant increase of these values - up to 2.65 for lead, for cadmium up to 17.35 and for zinc up to 4.88. Only plants which have values of the BAC and the TF greater than 1 have the potential to be used for phytoextraction.

Table 5. Uptake of heavy metals from safflower (Bioaccumulation factor (BAC, EF)

Element	Control	Compost		Vermicompost	
		20 t/daa	40 t/daa	20 t/daa	40 t/daa
Pb	1.30	0.99	1.27	1.80	2.65
Cd	10.23	7.34	9.21	10.28	17.35
Zn	3.53	2.08	2.16	2.79	4.88

$$BAC\ (EF)=[Metal]_{abobe\ ground\ part}/[Available\ metal]_{soil}$$

Therefore, in order to be classified as a hyperaccumulator, a plant must have values of the EF and the TF > 1. The values obtained for the BCF and the TF are the most important test that can be used to assess the potential of plants to be used for phytoremediation. Our results indicate that both factors EF and TF of the safflower from the experimental control are greater than 1, and according to this indicator, it can also be classified as a hyperaccumulator of cadmium.

Given the values of the TF about the capacity of safflower to transport contaminants from the soil and the value of the BAC, it can definitely be argued that safflower can transport and accumulate not only cadmium, but also lead and zinc after introduction of 40 t/daa of vermicompost to the soil.

Lasat (2000) and Li et al. (2003) note the importance of soil fertility for raising the level of phytoextraction of metals. Our results confirm that the introduction of organic ameliorants into the soil leads to a more efficient movement of elements in comparison with the control. Similar are the results of Marchiol et al. (2007), according to which fertilization leads to the production of more biomass and to a more efficient removal of contaminants from the soil. Best performance of the process can be achieved by the use of plants with a large biomass and value of the bioconcentration factor > 1.

Comparison of the criteria shows that, in terms of cadmium, safflower covers all the criteria necessary for it to be assigned to the hyperaccumulators of Cd. In terms of lead and zinc, the plant does not cover only 1 of the criteria - sufficient accumulation of metals in the aboveground biomass (less than 1000 mg/kg for Pb and 10000 mg/kg for Zn) and so safflower can be assigned to the group of accumulators of lead and zinc.

Introduction of vermicompost increases the potential of safflower to absorb heavy metals, while introduction of compost does not result in a significant impact on the capacity of the plant in this respect.

Although phytoremediation is a new technology, in recent years many studies have been conducted in an attempt to find out how plants absorb larger quantities of metals, the mechanisms of movement and translocation of the metals from their roots to the aboveground biomass, and their storage and detoxification. One of the main principles of phytoremediation is

to find suitable plant species that can be grown on contaminated soil. Our results strongly suggest that safflower is a crop which is tolerant to heavy metals and can be grown in contaminated soil. It can be assigned to the hyperaccumulators of cadmium and to the accumulators of lead and zinc, and therefore, it can be successfully used for phytoremediation of soils contaminated with Cd.

Safflower is a crop with a deeply penetrating root system and a high annual production of biomass (Weiss, 2000). It can be assigned to the energy crops for production of biodiesel, which is obtained in the production of oil from its seeds (Sims et al., 2006).

It is necessary to carry out further research in order to clarify the real potential of safflower when grown on larger areas because there is no practical experience from the cultivation of this crop in our country. Experts will need to offer additional management practices in order to increase the potential and efficiency of safflower for cleansing soil of heavy metal contaminants. The cultivation of the plant, planting, irrigation, pests and diseases, crop rotation, cycle duration and harvesting should be further studied. For now there is no experience in the cultivation of safflower in Bulgaria under field conditions, especially on heavy metal contaminated soils. These issues need further clarification and a significant amount of research.

4. CONCLUSIONS

Based on the results obtained we can make the following important conclusions:

1. The introduction of organic additives in the soil affects the physico-chemical properties and leads to an increase in the organic matter, the total N, the electrical conductivity, and the content of macroelements (P, K, Ca and Mg) in the soil, and this increase depends on the composition of the organic additive.
2. Organic ameliorants reduce the amount of the DTPA-extracted mobile forms of Pb and Cu, and the decrease is proportional to the amount of the introduced additive. The introduction of vermicompost lowers the content of the mobile forms of Zn and Cd, while the compost leads to their increase.
3. The results of the present study indicated that soil application of compost and vermicompost in most cases decreased DTPA-extractable levels of heavy metals in the soil. This is indicative of heavy metal immobilization by humic substances from compost and vermicompost application. The results appear to verify the function of humic acid in improving phytoremediation efficiency of soils contaminated with heavy metal and potential environmental availability of metals may be controlled by soil organic amendments.
4. The distribution of heavy metals in the organs of the safflower has a selective character that decreases in the following order: leaves > roots > stems > seeds.The regenerative organs of safflower, when being cultivated on heavy metal contaminated soils, accumulate Cd at levels significantly above the recommended maximum levels tolerated by the animals. The introduction of compost and vermicompost has a significant effect on reducing the heavy metal content in the seeds of safflower. The introduction of 40 t / daa of compost and 40 t/ daa of vermicompost leads to the reduction of Cd to levels of 2.9 mg/kg to 0.5 mg/kg, which is within the maximum limits for forage.

5. Safflower is a plant which is tolerant to heavy metals and can be grown on contaminated soils, and which can be referred to as the hyperaccumulators of cadmium and the accumulators of lead and zinc, and can be successfully used in the phytoremediation of heavy metal contaminated soils. The processing of seeds to oil and using the obtained oil for nutritional purposes will greatly reduce the cost of phytoremediation.

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DIGESTION METHOD TO DETERMINE METAL IMPURITIES IN WIPE SAMPLES OF CARBON NANOTUBES

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ABSTRACT

Carbon nanotubes (CNTs) contain metal impurities (e.g. Mo, Ni, Co, Y, Fe) which are introduced during the manufacturing process (carbon structure catalytic growth). Therefore, the detection of CNT-bound metal impurities in environmental samples provides a means to identify the presence of CNT particles. Quantification of CNT-bound metal impurities poses a significant analytical challenge since metals at different concentration levels (i.e. ppm or ppb) may be firmly incorporated in the carbon structure. The purpose of this study is to identify digestion methods that in conjunction with ICP-MS determination will provide the best quantitative estimates of metal impurities in CNT particles sampled from surfaces using GhostWipes. While Inductively Coupled Plasma Mass Spectrometry (ICP-MS) is useful for multi-elemental determination of metals in various matrices, in this case it is limited by the difficulty in quantitatively extracting CNT metal impurities during the digestion step.

Three digestion methods using nitric acid and hydrogen peroxide were evaluated during this study: microwave, hot-block, and a combined hot-block/microwave digestion method. Aliquots of NIST 2483 Single Wall CNT Standard Reference Material (SRM) were combined with GhostWipes to compare the efficiency of investigated digestion methods. GhostWipes are composed of polyvinyl alcohol copolymer and meet the ASTM E-1792 wipe sampling standard. A preliminary semi-quantitative scan of the digests identified Ge, In and Re as appropriate internal standards for NIST 2483. Recoveries of 87% and 110% respectively were obtained for Co and Mo in NIST 2483 using the combined hot-block/microwave method. Recoveries for the same SRM were less efficient using hot-block digestion alone (Co 49%; Mo 91%) and using microwave digestion alone (Co 35%; Mo 80%, respectively). These results indicate that the combined hot-block/microwave digestion method may be necessary to quantitatively extract metal impurities from CNTs using nitric acid and hydrogen peroxide.

Keywords: Carbon nanotubes, exposure sampling, hot-block digestion, microwave digestion, ICP-MS, GhostWipes, metals.

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1. INTRODUCTION

Carbon nanotubes (CNTs) are produced by various processes (i.e. electric arc discharge, laser evaporation, and catalytic decomposition of hydrocarbons) with different catalysts employed in their synthesis (ISO/TS 13278: 2011). Depending on the manufacturing process, CNTs contain different metal impurities (e.g. Fe, Co, Mo, Ni, and Y) embedded in their graphitic structure at different concentration levels (Adeleye and Keller 2014; ISO/TS 13278: 2011; Mwangi et al. 2012; Yang et al. 2010). Monitoring those residual metals as tracers for CNT presence was proposed as a useful alternate strategy to distinguish process-related CNT emissions from background (AIST 2013; NIOSH 2013; Rasmussen et al. 2013). Among other sampling methods (i.e. filter cassettes, electrostatic precipitations) the use of surface wipes has been suggested as a possible strategy to collect samples for workspace monitoring of CNTs (Rasmussen et al. 2013).

The purpose of this study is to identify digestion methods that in conjunction with Inductively Coupled Plasma Mass Spectrometry (ICP-MS) determination will provide the best quantitative estimates of metal impurities in CNT particles collected from surfaces using GhostWipes. While ICP-MS is useful for multi-elemental determination of metals in various matrices and recommended for analysis of metals in CNT digests (Decker et al. 2009), in this case it is limited by the difficulty in quantitatively extracting CNT metal impurities during the digestion step. Different studies found that microwave digestion may result in incomplete extraction possibly due to inefficient contact between particles and acid caused by the higher solid:liquid ratio and absence of agitation in the microwave method (Rasmussen et al. 2013), and that the application of repeated microwaving heating cycles with new reagent addition might be necessary for acceptable results (ISO/TS 13278: 2011). Ultrasonic digestion with a strong acid mixture (HNO_3-HF) has been used for a similar matrix (NIST 1633b coal fly ash) with recoveries of 80–120% for almost all of the 20 elements tested (Niu et al. 2013). McDonald et al. (2011) used hot-block digestion with a strong acid mixture (HF-HNO_3-H_2O_2) for multielement analysis of residential dust collected on GhostWipes in accordance with the ASTM E-1792 wipe sampling protocol. However, as the use of HF is avoided in many laboratories for safety reasons, an alternative acid digestion mixture is required that yields acceptable recoveries without the use of HF.

For the purpose of this study, NIST 2483 Single Wall CNT was digested with one or two GhostWipes (composed of polyvinyl alcohol copolymer) to evaluate the efficiency of the proposed method for surface samples. Subsamples of NIST 2483 combined with GhostWipes were subjected to hot-block digestion followed by microwave digestion, using a combination of nitric acid and hydrogen peroxide. For the purpose of method optimization, recoveries using this combined Hot-Block/Microwave Digestion procedure (HBMW) are then compared with recoveries using hot-block digestion alone (HB) and microwave digestion alone (MW).

2. MATERIAL AND METHODS

2.1 Samples

GhostWipes (15cm x 15cm; Environmental Express, Charleston, South Carolina, SC 4250) come pre-moistened with deionized water in individually sealed packets. During the analytical procedure, the GhostWipe dissolves completely allowing the sampled material/analytes to

disperse in the digestion media (www.envexp.com). Proposed protocols for CNT sampling in workplace environments may use one or two GhostWipes to collect samples.

GhostWipes (GW) were combined with NIST 2483 Single Wall CNT Standard Reference Material (SRM) in the following experiments to compare the efficiency of investigated digestion methods when the SRM is in the presence of the GW matrix. For all methods, five replicates of 2 mg of SRM were quantitatively transferred to digestion vessels containing one or two GW. Aliquots of SRM were also analysed without GW. Spiked samples were prepared using SRM, GW and appropriate masses of enriched spikes (100 ppb level). Procedural blanks with and without GWs and spiked GW blanks (10 ppm level) were also analyzed along with the samples.

2.2 Chemical Reagents

High purity nitric acid (SEASTAR Chemicals Inc, Sidney BC, UN2031, CAS 7697-37-2) and Suprapur 30% aqueous solution of hydrogen peroxide were used for sample preparation. Ultrapure Milli-Q water (18.2 MΩ cm) was used for preparation of samples and calibration standards. High purity standard stock solutions (Delta Scientific Laboratory Products Ltd., Mississauga, ON) were used to prepare the calibration standards as follows: MES-1107-01 Solution A (100 µg/mL Al, As, Ba, Be, Bi, B, Cd, Cr, Co, Cu, Fe, Pb, Mn, Ni ,Se, Sr, U, V, Zn, in 2 % HNO3), MES-1107-01 Solution B (100 µg/mL Sb, Mo, Ag, Sn, Ti in 2 % HNO3 + TrHF) and individual high-purity standards solutions of La and Gd (1000 µg/mL in 5% HNO_3). All standards were prepared in 1% HNO_3 to match the matrix of the samples. GhostWipes blanks and SRM replicates were spiked at levels varying between 10 µg/L and 100 µg/L, respectively, with ICP-MSCS high-purity standard solution (10 µg/ml Al, Sb, As, Ba, Be, B, Cd, Ca, Cr, Co, Cu, Eu, Ho, La, Pb, Li, Mg, Mn, Mo, Ni, Sc, Se, Ag, Na, Sr, Tl, Th, U, V, Yb, Zn in 2% HNO_3+ TrHF). Individual high-purity standards solutions of Ge, In, and Re (1000 mg/L in 2% HNO3) were used to prepare the internal standard solution (25 µg/L Ge, In, Re).

2.3 Digestion Procedures

A combined hot-block/microwave digestion method (HBMW), using nitric acid and hydrogen peroxide was optimized to extract the CNT-bound impurities in the presence of GhostWipes. The developed method presented in this study consists of two separate steps: step 1 consists of wet digestion using a DigiPrep digestion block (HB) followed by step 2, which consists of microwave digestion (MW) of the digestate generated in step 1.

Step 1: Hot-Block digestion (HB). Twelve mL of diluted nitric acid (1:1 nitric acid to water) was added to each 50 mL Teflon DigiTube digestion vessels. Samples were let to stand for 30-45 min until the GhostWipes dissolved completely and effervescence subsided. Another portion of 12 mL diluted nitric acid (1:1 nitric acid to water) was added to each tube and the samples were transferred to DigiPrep MS heating block and gently heated at approximately 85°C. With a programmed automatic shut-off, DigiPrep MS block allows monitoring of both the temperature and the volume of solution. After 90 min reflux without boiling, 10 mL of concentrated HNO_3 was added and the heating continued at reflux for another 30 minutes. After evaporation to approximately 10 mL at 90°C and cool down to room temperature, 5 mL of 30% hydrogen peroxide was added to each tube and covered tubes were returned to the DigiPrep heating block. The solutions were gently heated at 85°C and then refluxed for 50 min until the effervescence

subsided. The heating of the acid-peroxide digestate continued carefully at 90°C until the volume reduced to approximately 10 mL. This step is modified after McDonald et al. (2011).

Step 2: Microwave digestion (MW). The solutions were then quantitatively transferred to high-pressure, pre-cleaned 100 mLTeflon digestion vessels and 0.5 mL of 30% hydrogen peroxide was added to each vessel. The vessels were sealed and placed in a Ethos Touch Control Advanced Microwave Labstation (Milestone Microwave Laboratory Systems), equipped with Ethos TC built-in ATC-400-CE automatic temperature control. Microwave digestion was performed at 1000W power in the following conditions: 20 min ramp to 180^{o}C, 10 min ramp from 180^{o}C to 220^{o}C, and 20 min hold at 220^{o}C. After the microwave program ended the vessels were allowed to cool to room temperature. Each digested solution was quantitatively transferred to clean 50 mL DigiTubes and evaporated to dryness at 85^{o}C. The resultant residues were dissolved in 0.25 mL nitric acid and diluted to 25 mL with high-purity water. After filtration through a 0.45 μm filter in cleaned DigiTubes to remove the possible undissolved residuum that may impact the ICP-MS analysis, sample solutions were stored in the fridge and analysed by ICP-MS (see section 2.4). This step was developed based on methods described in Decker et al (2009) and Hassan et al (2007).

HBMW method optimization. To shorten the time of the procedure, variations of the combined method (HBMW) were investigated by eliminating either the hot-block step (resulting in a microwave alone digestion: MW) or the microwave step (resulting in a hot-block alone digestion: HB). The MW digestion alone was done by digesting aliquots of NIST 2483 combined with GW with 6 mL HNO_3, 0.5 mL H_2O_2 and 3.5 mL ultrapure water and following the protocol described in step 2. Recoveries were calculated for each method/trial and the advantages and disadvantages were considered.

2.4 ICP-MS Analysis

For quantification of CNT metal bound impurities in digested samples, NexION 300s with Dual-channel Universal Cell ICP-MS system (Perkin Elmer, Canada) equipped with a SC-Fast autosampler (Elemental Scientific, Omaha, NE), a high temperature apex-ST PFA MicroFlow nebulizer, cyclonic spray chamber and a PC3x chiller operated at 2^{o}C, and a triple cone interface (nickel-platinum skimmer and sampler cone, and aluminium hyper cone) was operated in the standard mode for all elements except Fe for which reaction mode was used. The following conditions were used: plasma and auxiliary argon flow rates were 18 and 1.2 L/min, respectively. The nebulizer argon gas flow rate was 1L/min for the high temperature apex-ST PFA MicroFlow concentric nebulizer. The forward RF power was 1600 W. Optimization was carried out daily with a normal tuning solution (1 ng/mL Be, Ce, Fe, In, Li, Mg, Pb, U). Based on a preliminary semi-quantitative analysis the appropriate dilution factors were determined and the following internal standards were selected: Ge, In and Re. Three replicate readings were taken for all monitored masses and elements. Spike recoveries of the study elements (B, Al, V, Co, Mo, As, Ba, La) were between 93% and 115%. Limits of detection (LOD) are reported in Table 1.

3. RESULTS AND DISCUSSION

Table 1 presents the concentrations (mean and standard deviation; μg/g) for metal impurities in NIST 2483 with GhostWipes obtained using the combined hot-block/microwave method

(HBMW), microwave digestion alone (MW) and hot block digestion alone (HB). The results were corrected using corresponding GhostWipe blank values.

The main metal impurities in NIST 2483, as shown in the certificate of analysis and included in Table 1, were cobalt and molybdenum with certified mass fraction values of 9630 ± 170 µg/g and 34060 ± 290 µg/g for Co and Mo, respectively, determined by instrumental neutron activation analysis (INAA) and cold neutron Prompt Gamma Activation Analysis (PGAA). Certified values for barium, gadolinium, and lanthanum which were present in smaller quantities in NIST 2483 are also included in Table 1. Reference values are provided for aluminium, manganese and vanadium and informational values are provided for other metals in Table 1 (arsenic, boron, and copper).

Table 1 indicates that HBMW yielded better recovery for both Co and Mo, the high concentration metals in NIST 2483, than either of the two shortened versions. Digestion efficiencies of the short methods were lower by factors of 2.5 (MW) and 1.8 (HB) for Co and 1.4 (MW) and 1.2 (HB) for Mo (Table 1). Also, the combined method extracted higher amounts of As (11.9 µg/g) than the microwave method (2.88 µg/g) and the hot-block method (not detectable), and comparable amounts of La (97.6 µg/g HBMW, 71.4 µg/g MW, and 85.8 µg/g MW, respectively), Ba (79.1 µg/g HBMW and 75.1 µg/g MW) and Gd (11 µg/g HBMW, 9.28 µg/g MW and 9.82 µg/g HB). In the case of Ba, recoveries for the HB method were lower by a factor of 1.5 compared to the other methods (HBMW or MW).

Table 1. Concentrations of metals recovered from NIST 2483 in combination with 2 GhostWipes using the combined method (HBMW), microwave alone (MW), and hot-block digestion alone (HB). The results are presented as mean and standard deviation of five independent determinations except for microwave (n=3). nd = not detectable after GhostWipe blank correction.

Element	LOD (µg/g)	Certified value (µg/g)	HBMW (µg/g, n=5)	MW (µg/g, n=3)	HB (µg/g, n=5)
Cobalt	2.43	9630 ± 170	8347 ± 1134	3320 ± 797	4696 ± 154
Arsenic	0.15	12.5	11.9 ± 4.40	2.88 ± 2.19	nd
Molybdenum	2.71	34060 ± 290	37618 ± 4025	27244 ± 7369	30988 ± 648
Barium	1.19	119 ± 3.4	79.1 ± 16.4	75.1 ± 10.8	53.8 ± 17.5
Lanthanum	0.15	104 ± 4	97.6 ± 9.38	71.4 ± 7.44	85.8 ± 2.20
Gadolinium	0.04	10.57 ± 0.95	11.0 ± 1.13	9.28 ± 0.95	9.82 ± 0.29
Boron	9.72	74.7	*nd	*nd	*nd
Aluminum	431	723 ± 19	*415 ± 433	*nd	*nd
Vanadium	0.17	6.89 ± 0.14	*nd	*nd	6.38 ± 2.68
Copper	7.54	186	*nd	*nd	*746 ± 392

* poor recovery due to interferences (Cu in HB) and highly variable GW blanks (B, Al, V, and Cu)

Unacceptably high blank values were observed for B, Al, V, and Cu due to background contamination of GhostWipes associated with the manufacturing process, resulting in non-detects and/or poor recovery for these elements after blank correction (Table 1). GhostWipe blanks were typically less than 0.05 µg per wipe for Co, Mo, As, La, and Gd; between 0.06 and 0.36 µg per wipe for Ni, Ba, Ti, Cr, Mn, Sr, Pb; more than 1.16 µg per wipe for B, Al, V, Cu, and Fe; and as high as 51 µg per wipe for Zn. Background contamination of Ba (0.15 µg per wipe) made quantification of this metal difficult using GhostWipe sampling, but was considered acceptable for qualitative identification. B, Al, V, Cu and the other elements displaying high

contamination levels in the GhostWipes yielded unacceptable results and are not considered further in this paper.

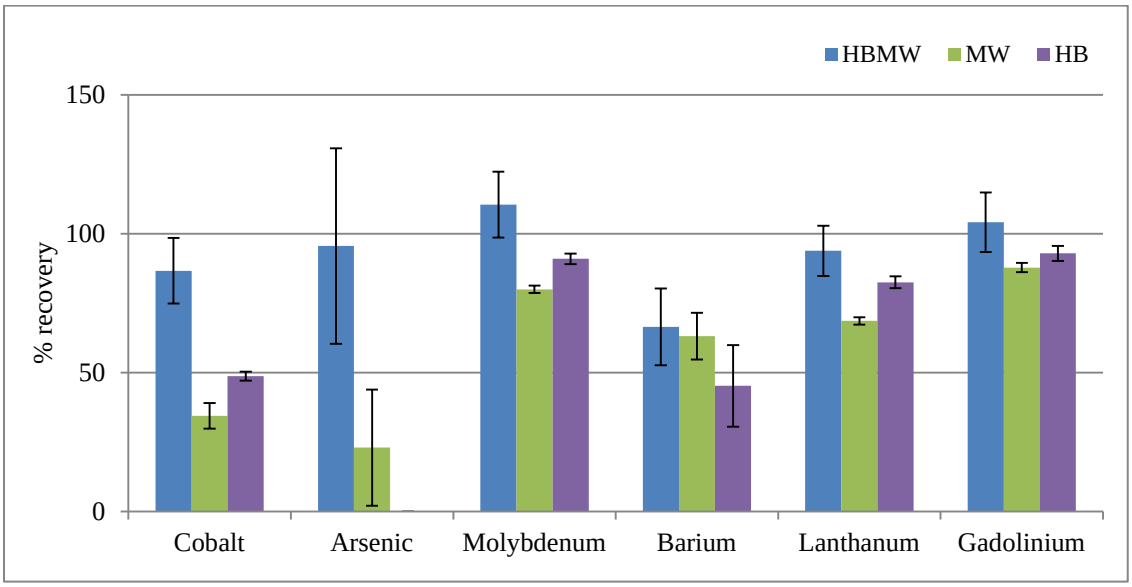

Figure 1. Percent recoveries of metals mobilized from NIST 2483 obtained with combined hot block/microwave digestion (HBMW, blue), microwave digestion (MW, green), and hot-block (HB, purple) digestion methods. The results are presented as mean and standard deviation of five independent determinations except for microwave (n=3).

Attempts to shorten the HBMW digestion by using microwave digestion alone or by using hot-block digestion alone both resulted in decreased extraction efficiency for metals in NIST 2483. This is illustrated for the six target metals in Figure 1, which compares percent recoveries using the combined hot block/microwave digestion (HBMW), microwave digestion alone (MW), and hot-block digestion alone (HB). Recoveries obtained with the HB method were similar to those of HBMW for Mo, La and Gd, but were 1.8 times lower for Co and 1.5 times lower for Ba (and below detection for As). The MW method yielded in comparable recoveries for Mo, Ba, La and Gd compared to HBMW, but four times lower recovery for As and 2.5 times lower recovery for Co (Figure 1).

Although previous studies (e.g. Decker et al. 2009; ISO/TS 13278: 2011) recommend the use of microwave digestion for determination of metal impurities in CNTs, Fig 1 shows that the extraction efficiency of MW compared to HBMW differs from metal to metal. Decreases in recovery of 76% for As, 60% for Co, 28% for Mo, 27% for La, 16% for Gd, and 5% for Ba were observed for MW compared to the HBMW method presented in this study (Figure 1). It appears that a longer contact time with the extraction acid combined with steps of hydrogen peroxide oxidation are required to expose and efficiently extract the encapsulated metal impurities from the CNT matrix, as in the combined hot-block/microwave method. Improved recoveries for CNT metal impurities may be obtained with microwave digestion by repeating the heating cycles several times until nearly complete digestion is achieved, as recommended by ISO/IEC 13278:2011 (i.e three to six heating cycles). Adding the preliminary HB digestion step, as recommended in the present study, eliminates the need for repeated heating cycles during the MW step, and has the added advantage of controlling the dissolution of the bulky GW matrix.

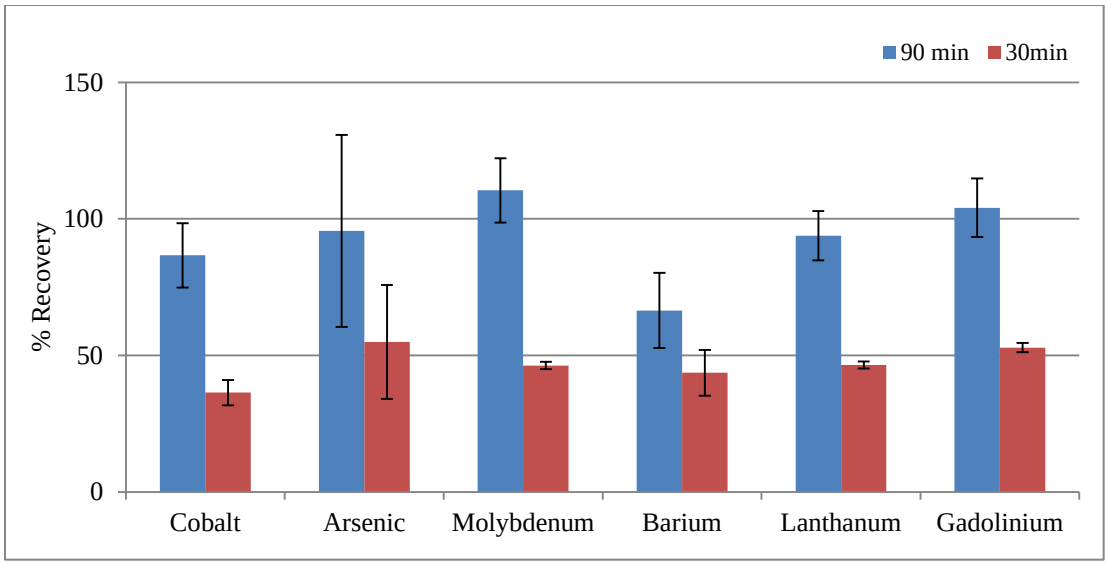

Figure 2. Decrease in recovery of metals from NIST 2483 caused by shortening the initial acid reflux stage from 90 min (blue) to 30 min (red). The results are presented as mean and standard deviation of five independent determinations. Error bars = 1 std dev for n=5.

Figure 2 illustrates another attempt to shorten the HBMW digestion by reducing the time of the first reflux with diluted 1:1 nitric acid from 90 min to 30 min. Reducing this step resulted in a 2.4-fold decrease in recovery for Co and Mo and a nearly two-fold decrease in recovery for Ba, La, As and Gd (Figure 2). This demonstrates once more the need for adequate contact time with nitric acid during reflux to expose the matrix-encapsulated metal catalysts. Methods for purifying CNTs similarly call for successive steps of oxidation, nitric acid reflux, and dissolution for complete extraction and removal of metal impurities (Goto et al. 2006).

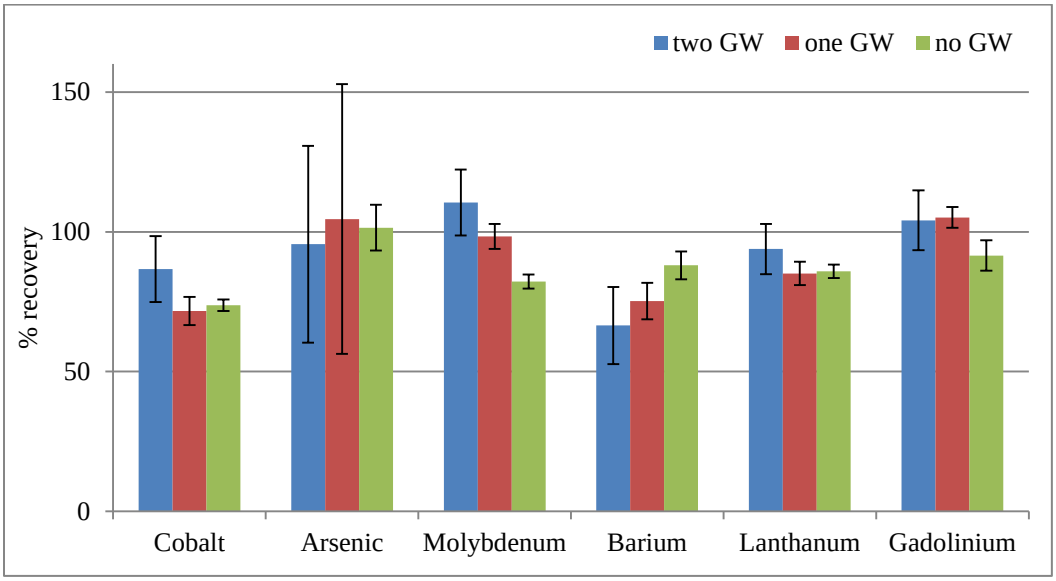

Figure 3. Mean recoveries obtained with HBMW method for metal impurities of NIST 2483 SWCNT with two GhostWipes (GW; blue), with one GW (red) and without GW in the digest matrix (green). Error bars = 1 std dev for n=5.

Finally, to evaluate the impact of GhostWipes on the extraction efficiency of the HBMW method for the target elements, 2 mg aliquots of NIST 2483 CNT were analysed without GW and in presence of one or two whole GW (Figure 3). Recoveries were between 72-87% for Co, 66-88% for Ba, and 80-110% for As, Mo, La, and Gd, indicating that one or two GWcan be tolerated in the HBMW digestion process for these elements. Overall, low variability of Co and Mo concentrations was observed with the HBMW method with relative standard deviations (RSD %) between 7-14% for Co and 5-11% for Mo (for one and two GWs). These RSDs are higher than the certified RSDs for NIST 2483 (Table 1) which may be related to the small sample mass used in this study (2 mg compared with 25-40 mg recommended in the certificate of analysis) and to the uncertainty associated with the GW blank correction.

4. CONCLUSION

Results indicate that a combined hot-block/microwave digestion (HBMW) may be necessary for quantitative extraction of metal impurities from CNTs using nitric acid and hydrogen peroxide (and avoiding the use of HF). The HBMW digestion is equally efficient for the extraction of metals from NIST 2483 CNT collected using GhostWipes, even when two GhostWipes are present in the digest matrix, with the exception of metals present as contaminants in the GhostWipe blanks (e.g. Zn, Al, and B). Attempts to shorten the digestion method by eliminating the hot-block step or the microwave step resulted in a significant decrease in recovery for some metals in NIST 2483, especially Co (from 87% recovery with HBMW to less than 50% recovery if either of the two steps is omitted). These results indicate that both hot-block digestion and microwave digestion are required to maximize the extraction efficiency of metals from the CNT graphitic structure. Each of the component methods, hot-block (HB) or microwave (MW), can be successfully applied for some metals, but it must be recognized that the efficiency of the selected method will differ from metal to metal as presented in this study. The increased contact time with the extraction acid in the HBMW method, plus the repeated oxidation and reflux stages, exposes the CNT encapsulated metals and removes the amorphous carbon mobilizing the metal impurities from the CNT graphitic structure into solution for quantification by ICP-MS. It is concluded that the HBMW digestion method will prove useful for determination of metal tracers in CNT samples collected on GhostWipes used for surface sampling in work environments.

5. ACKNOWLEDGMENTS

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ADDRESSING REMEDIATION OF POLYCYCLIC AROMATIC HYDROCARBONS AT A RECREATIONAL SITE IN NEW JERSEY

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds containing hydrogen and carbon, and they are resulting from incomplete industrial or household combustion of fossil fuels, diesel fuel and forest fires. PAHs are found in large quantities in coal tar, soot and creosote. PAHs are produced also during cigarette smoking and food grilling. PAHs are found almost everywhere and they pose a risk to human health because they are carcinogenic and bioavailable in water, soil, sediment and air, media that humans come in contact with daily.

This paper describes the process to develop a site-specific risk-based remediation standard for PAH for a municipal park / recreational use area in New Jersey. The state of New Jersey does not promulgate standards specifically to address recreational areas, but instead relies on the direct contact standards established for residential sites.

The analysis relied on a modification of the site-use exposure frequency used in calculating site risk for the residential direct contact standards, while adjusting for changes in the parameter quantification level (PQL) if applicable.

The analysis incorporated the New Jersey default risk tolerance level of one per million (1E-6), and specifically focused on benzo(a)pyrene (BaP), which is commonly a driving compound in remediation at sites in New Jersey.

The result demonstrates that an upward adjustment of the PAH remediation level for recreational use areas is justifiable, and remains protective of human health.

Keywords: benzo(a)pyrene, exposure frequency, risk assessment, recreational, park

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds containing hydrogen and carbon, and they are resulting from incomplete industrial or household combustion of fossil fuels, diesel fuel and forest fires. PAHs are found in large quantities in coal tar, soot and creosote. PAHs are produced also during cigarette smoking and food grilling.

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PAHs are found almost everywhere and they pose a risk to human health because they are carcinogenic and bioavailable in water, soil, sediment and air, media that humans come in contact with daily (USEPA [updated 2007], Wick et al. 2011 and (NJDEP [updated 2012]).

Historic fill material is common throughout New Jersey and commonly contains PAHs, including Benzo(a)pyrene (BaP). Sampling at a study site, a park, confirmed the presence of historic fill throughout the site, and showed the presence of BaP related to the historic fill in the shallow soils (upper 0-2 feet) in excess of New Jersey Department of Environmental Protection (NJDEP) Residential Direct Contact (RDC) Soil Remediation Standards (SRS) of 0.2 mg/kg (NJDEP 2012).

BaP exceeded the NJDEP RDC SRS of 0.2 mg/kg at multiple locations on the site, with concentrations ranging from Non-Detect (ND) to 3.85 mg/kg.

As required by NJDEP, the shallow BaP contamination was delineated to the RDC SRS in areas where no evidence of historic fill was present.

Risk assessment calculations were performed to evaluate a site-specific BaP Park Direct Contact (PDC) soil remediation standard, considering the park usage. A BaP Alternative Remediation Standard (ARS) of 0.44 mg/kg was determined by using an adjusted exposure time based on the site use.

The protectiveness of the proposed BaP PDC ARS was confirmed by comparing it with (1) published background levels in urban New Jersey public parks, (2) recreational standards available from the other state's environmental protection agencies, (3) RDC SRS derived by the other state's environmental protection agencies, and (4) the findings of a March 2012 New Jersey Department of Health (DOH) Letter Health Consultation (LHC) for a New Jersey park.

The figure below shows results of the BaP at the study site, compared to the NJDEP RDC SRS and the PDC ARS.

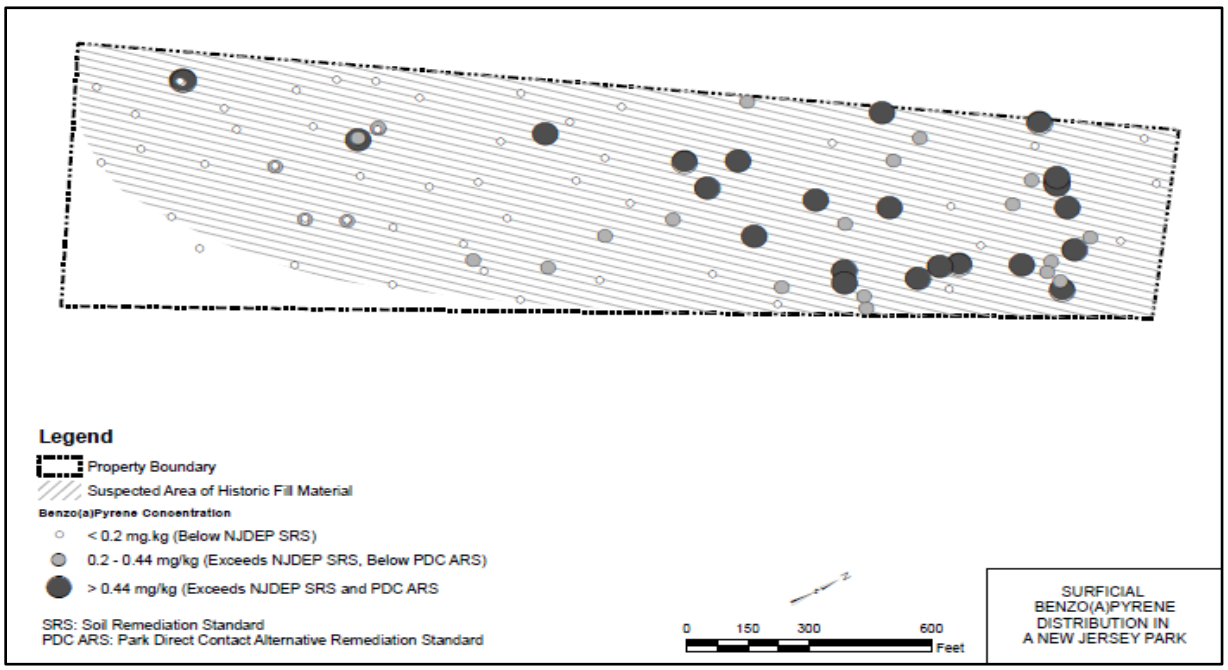

Figure 1. Surficial BaP Distribution at the park Study Site

2. RISK ASSESSMENT ANALYSIS

The current NJDEP RDC SRS for BaP is 0.2 mg/kg, and is based on a practical quantification limit (PQL), with a risk of 3.2E-6.

The RDC SRS are calculated by regulatory agencies using an exposure frequency (EF) of 7 days a week and 50 weeks per year (350 days), using the formula and input parameters listed below.

Remediation Standard =

$$\frac{TR * AT * 365 \frac{\text{days}}{\text{year}}}{\left(\frac{EF}{1{,}000{,}000}\frac{\text{kg}}{\text{mg}}\right) * \left[\left(SF_O * IF_{soil/adj/age}\right) + \left(SF_{abs} * SFS * ABS_d * EV\right)\right]}$$

Where,

TR is the target cancer risk (unitless) – TR is mandated 1E-6 in New Jersey, but for BaP, since the PQL is used in calulations, TR is defaulted to 3.2E-6;

AT is the averaging time – 70 years;

EF is the exposure frequency – 350 days/year;

SF_o is the oral cancer slope factor – chemical specific $(mg/kg\text{-}d)^{-1}$;

$IF_{soil/adj/age}$ is the adjusted soil ingestion factor – 114 mg-yr/kg-day;

SF_{abs} is the dermally adjusted cancer slope factor;

$$SFabs = \frac{SFo}{ABSgi} (mg/kg\text{-}day)^{-1}$$

ABS_{gi} is the gastrointestinal absorption factor – chemical specific (unitless);

SFS is the age-adjusted dermal factor – 360 mg-year/kg-event;

ABS_d is the dermal absorption fraction – chemical specific (unitless); and

EV is the event frequency – 1 event/day.

The EF value of 350 days/year is deemed appropriate for a residential property.

For a recreational park scenario, a reduced exposure was calculated using an EF of 1 day a week and 50 weeks per year - a total of 50 days/year, which was considered to be more appropriate since it accounts for the area's usage.

To back-calculate the BaP PDC ARS, a risk of 1.0E-6 was used, which is more protective than the target cancer risk (TR) for BaP RDC SRS of 3.2E-6.

Using the anticipated park exposure frequency of 50 days/year, the TR of 1E-6, and keeping all the other factors the same, as in the RDC SRS scenario, a BaP PDC SRS of 0.44 mg/kg was calculated.

Alternately, if we used the TR based on the PQL of 3.2E-6, and an equivalent allowable exposure frequency of 150 days per year, that corresponds to 3 days a week for 50 weeks, a similar BaP PDC ARS is calculated, as shown in Table 1 below.

These approaches are consistent with United States Environmental Protection Agency guidance (USEPA 2004), which specifies the residential direct contact to be event based.

Table 1. Input parameters for Bap PDC ARS

Parameter	**Unit**	**7 days/week**		**1 days/week**	**3 days/week**
TR	**unitless**	**1.00E-06**	**3.20E-06**	**1.00E-06**	**3.20E-06**
AT	years	70	70	70	70
EF	**days/year**	**350**	**350**	**50**	**150**
SF_{abs}	$(mg/kg\text{-}day)^{-1}$	7.3	7.3	7.3	7.3
SFS	mg-year/kg-event	360	360	360	360
ABS_d	unitless	0.13	0.13	0.13	0.13
EV	Events/day	1	1	1	1
SF_o	$(mg/kg\text{-}d)^{-1}$	7.3	7.3	7.3	7.3
$IF_{soil/adj/age}$	mg-yr/kg-day	114	114	114	114
RDC SRS	mg/kg	0.06	0.2	0.44	0.46
Defaulted to PQL	mg/kg	0.2			

3. ADDITIONAL PROTECTIVENESS

3.1 Background Concentrations

Regional background BaP concentrations were evaluated from the NJDEP publication "Characterization of Ambient Levels of Selected Metals and Other Analytes in New Jersey Soils: Year 1, Urban Piedmont Region" (BEM 1997). The study determined background levels for the Urban Piedmont region, where historic fill material is common, by analyzing soil samples collected in public parks.

In this study, BaP was detected in 61 of 67 samples collected in the Urban Piedmont region, with an average concentration of 0.61 mg/kg, a 95th percentile concentration of 2.91 and an upper 95th percent confidence limit (UCL) concentration of 2.17 mg/kg.

The proposed BaP ARS of 0.44 mg/kg is well below the ambient BaP concentration determined by the NJDEP in Urban Piedmont parks.

3.2 Other State's Recreational Standards

Although the NJDEP publishes soil remediation standards only for residential and non-residential exposure scenarios, some other states additionally publish soil standards for recreational exposure scenarios.

For example, the Indiana Department of Environmental Management (IDEM) has calculated BaP recreational soil direct contact screening levels of 5 mg/kg for a trail, 3 mg/kg for an athletic field, and 1 mg/kg for a community park (IDEM 2012).

The New York State Department of Environmental Conservation (NYSDEC) applies to active recreational sites their BaP Restricted-Residential Soil Cleanup Objective of 1 mg/kg, which is based on the rural soil background levels (NYSDEC 2010).

Maine Department of Environmental Protection (MEDEP) has published a similar BaP soil screening level of 0.44 mg/kg for their parks (MEDEP 2013).

Again, the proposed BaP PDC ARS of 0.44 mg/kg is below, or equal to, the enforced BaP maximum concentration allowed in other states in recreational scenarios.

3.3 Other State's Residential Standards

A summary of the 50 United States (US) residential direct contact standards is presented in the figure below.

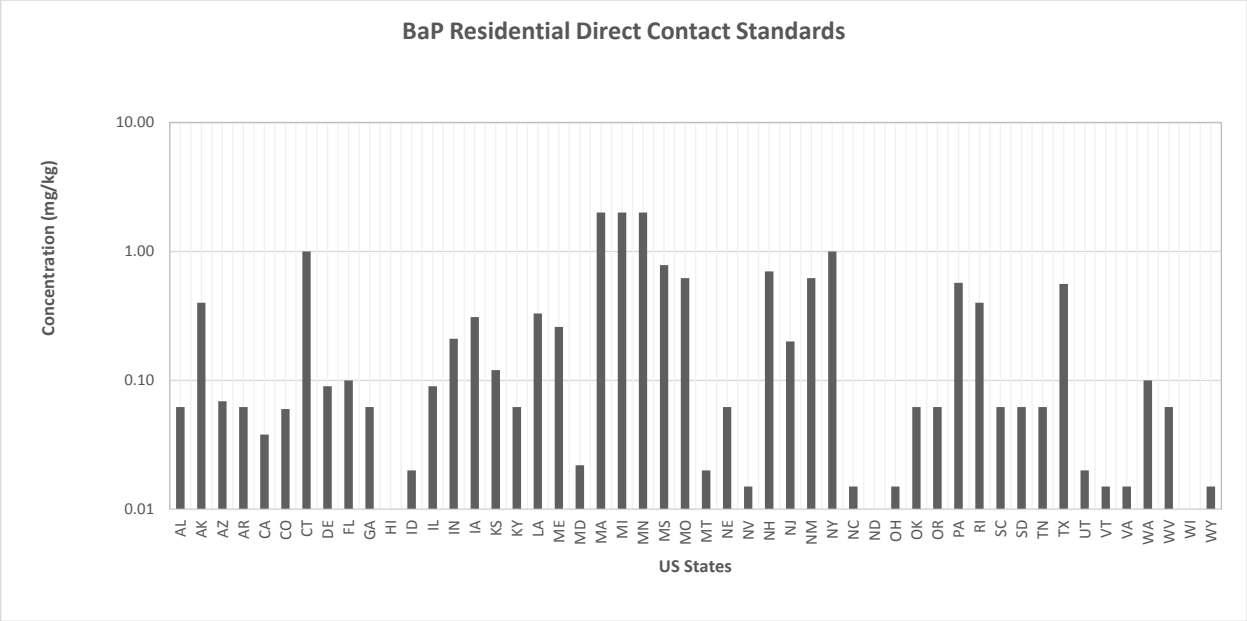

Figure 2. BaP Residential Direct Contact Standards

As it can be seen, the proposed BaP PDC ARS of 0.44 mg/kg is within the range of the BaP RDC SRS, of 0.015 mg/kg, in the states like Virginia (VADEQ 2014), Vermont (VTDEC 2012) and Wyoming (WYDEQ 2013) and 2 mg/kg, in states like Massachusetts (MassDEP 2014), Michigan (MIDEQ 2013) and Minnesota (Grosenheider et al. 2006).

3.4 NJ DOH Findings

In March 2012, the NJ DOH completed a Letter Health Consultation (LHC) for a New Jersey park at the request of the Health Department. The LHC evaluated surficial soil sample results to determine potential exposure risks to park users from direct contact with the soil.

The LHC concluded that, "past exposures to soil contaminated with PAH compounds […] at the […] park are not expected to harm people's health…In addition, PAH concentrations detected in surface soil at the park were consistent with background levels as evidenced upon comparison to studies of background PAH concentrations detected in surface soil for urban areas of New Jersey and other east coast states" (NJDOH 2012).

4. CONCLUSIONS

Surficial soil sampling at a recreational study site in New Jersey confirmed the presence of benzo(a)pyrene (BaP) related to historic fill at concentrations exceeding the NJDEP Residential and Non-Residential Direct Contact Soil Remediation Standard of 0.2 mg/kg.

A risk analysis was conducted using an adjusted exposure time to account for the recreational usage of the park. A Park Direct Contact (PDC) Alternative Remediation Standard (ARS) of 0.44 mg/kg was calculated for the park.

The PDC ARS relied on a risk of 1.0E-6, which is more protective than the PQL-based New Jersey Department of Environmental Protection (NJDEP) Residential Direct Contact (RDC) Soil Remediation Standard (SRS). Furthermore, the ARS is below both the established regional background concentration of BaP, and the residential and recreational standards in place in other states.

The findings of the risk assessment are also consistent with the findings of the NJ Department of Health (DOH) study conducted for polycyclic aromatic hydrocarbons (PAH) contamination in a New Jersey park.

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CHLOROETHENES DEGRADATION IN MICROCOSMS CONTAINING FERMENTABLE SUBSTRATE, NEAT ZVI, OR FERMENTABLE CARBON-ZVI MIXTURE

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ABSTRACT

A year-long bench test was undertaken to evaluate anaerobic degradation of tetrachloroethene to ethene in soil-groundwater slurries enriched with: 1) fermentable carbon substrate; 2) zero valent iron (ZVI) powder; or 3) a mixture of fermentable carbon substrate and ZVI powder. Microcosms containing fermentable carbon were augmented after six months with bacteria known to sequentially dechlorinate PCE to non-toxic ethene. Changes in dissolved chloroethenes concentrations are compared to changes in ethene, ORP, and pH.

pH is well recognized as a significant factor that controls rates of many biotic and abiotic reactions, including those known to dechlorinate chloroethenes. Many variables contribute to optimum pH for various dechlorinating pathways for chloroethenes; however, current general opinion is that biotic reductive dechlorination by *Dehalococcoides* spp. is stifled below about pH 6 (and inhibited below about pH 5), and strict abiotic dechlorination by zero valent iron is inhibited above about pH 9.

The results indicate that pH in a poorly-buffered environment can be induced to degradation rate-limiting or rate-inhibiting values under conditions of the test; pH too low in the case of amending only with fermentable carbon substrate, and pH too high in the case of amending with only ZVI. However, amending fermentable carbon substrate mixed with ZVI prevented pH from migrating into degradation rate-inhibiting territories, both low and high.

Keywords: Chloroethenes, Degradation, Microcosm, Carbon, ZVI, pH Inhibition.

1. INTRODUCTION

Mixed soil-groundwater slurry microcosm testing was conducted for a Superfund site in Rhode Island to evaluate the effectiveness of five (5) injectable reagents to stimulate biotic, abiotic, or microbially-mediated abiotic degradation of tetrachloroethene (PCE) and its degradation products, especially cis-1,2-dichloroethene, in each of the three types of soil encountered in the site's water-saturated unconsolidated aquifer.

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Soil types tested were:

- Peat
- Silt
- Sand and Gravel.

Reagents tested were:

- ABC: Anaerobic BioChem fermentable carbon substrate by Redox Tech; available through Carus.
- Peerless 50D Iron Powder: meso-sized zero valent iron (ZVI)
- ABC+: Anaerobic BioChem fermentable carbon substrate Plus meso-sized ZVI by Redox Tech; available through Carus.
- Hepure HCA-325 Iron Powder: micro-sized ZVI
- EHC-F: Eh Compound - Fine-grind fermentable carbon substrate blended with micro-sized ZVI by PeroxyChem (formerly FMC, formerly Adventus).

CB&I (formerly Shaw Environmental) Biotechnology Development and Applications Laboratory (Lawrenceville, NJ), under the direction of Dr. Robert Steffan, constructed, maintained, and analyzed the mixed slurry microcosms for 330 days (September 2012 to August 2013). DNA-based bacteria content testing was performed at Microbial Insights in Knoxville, TN.

2. MATERIALS AND PROCEDURE

Materials and methods used to manufacture and test the mixed slurry microcosms are described in the following sub-sections.

2.1 Soil Feedstock

Soil feedstock samples were collected at the locations shown on Figure 1.

Peat and Silt soil cores were collected with a Geoprobe 7822DT direct-push rig with 4.5-inch diameter tooling and Geoprobe DT45 Soil Sampling System, which returned nominal 5-foot long cores in 3-inch diameter polyvinyl chloride (PVC) liners. Sand and Gravel soil cores were collected with a Geoprobe 7822DT direct-push rig using with 3.5-inch diameter tooling and Geoprobe DT35 Soil Sampling System, which returned nominal 5-foot long cores in 1.85-inch diameter PVC liners.

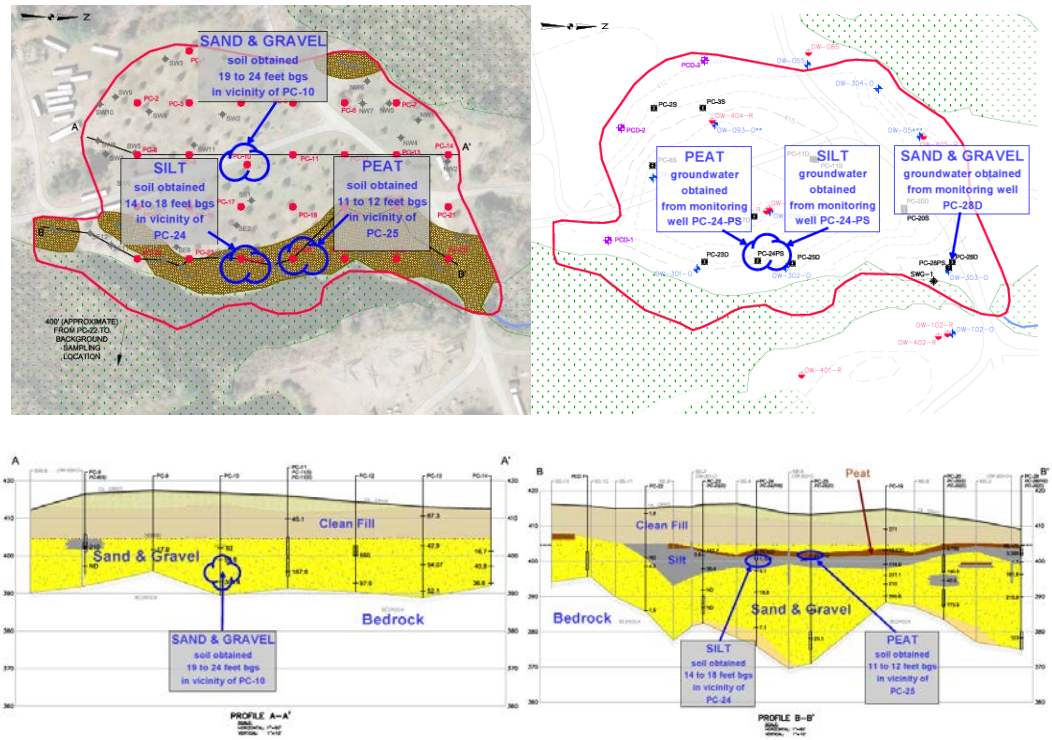

Figure 1. Soil and Groundwater Feedstock Sample Locations.

A minimum 6-inch section from each end of each core was severed before immediately capping each end with an airtight plastic cap. These nominal 4-foot long core samples were labeled according to target soil type, depth of collection, and a unique identifying number. Airtight, labeled, core samples were immediately placed horizontally in a dark ice-filled marine grade chest cooler atop a cushion of bubble wrap to prevent cold-shocking native bacteria. The intent was to quench further microbial activity by slow cooling to approximately 4 degrees Celsius (°C) without killing or changing the native bacteria consortium by preserving dark anaerobic conditions to which they are accustomed.

Soil core samples were delivered to the treatability lab by the bench test coordinator. The bench test coordinator assisted the laboratory staff with selecting qualifying materials for manufacturing of the mixed slurry microcosms. Examples of qualifying materials are shown in Figure 2.

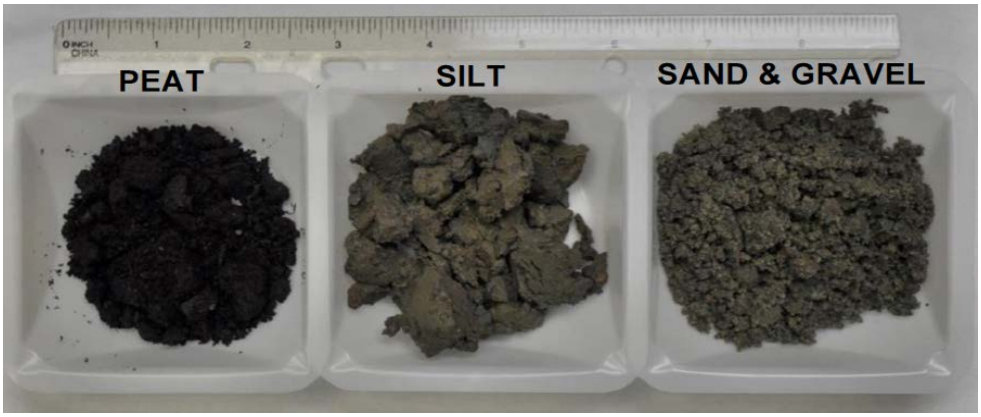

Figure 2. Typical Qualifying Soil Feedstocks.

The clear PVC casing of each soil core was scored (but not penetrated) lengthwise on opposing sides with a table saw so that the cores could be fully split in half with a razor knife after insertion into in a hydrogen free and anoxic glove box (aka anaerobic chamber). The top and bottom six inches of soil from each core was rejected. The bench test coordinator selected qualifying material in each core. Qualifying material from each core was placed into individual clean sterile glass jars and sealed with a Teflon-lined cap. Qualifying material for each soil type was then composited and homogenized into larger clean and sterile glass jars, sealed, labeled, and stored in the dark at 15°C until used to construct the slurried microcosm samples.

2.2 Groundwater Feedstock

Ten liters of qualifying groundwater was collected from monitoring well PC-24-PS as feedstock to manufacture slurries with Peat. Ten liters of qualifying groundwater was collected from monitoring well PC-24-PS as feedstock to manufacture slurries with Silt. Ten liters of qualifying groundwater was collected from monitoring well PC-28D to manufacture slurries with Sand and Gravel.

Groundwater samples were collected by a peristaltic pump and dedicated polyethylene tubing as per the Standard Operating Procedure for low-flow groundwater sampling. Great care was taken to assure that native bacteria in the groundwater were not compromised due to oxygen toxicity or by light or temperature shock. In-well drawdown was constantly measured to assure the level never fell below the well's upper screen level (prevent oxygenation by trickle-down). Pumped water was constantly monitored in a flow-through cell for dissolved oxygen, pH, oxidation/reduction potential (ORP), and temperature. New, clean, and preservative free 1-liter amber glass bottles were bottom up purged with nitrogen gas before filling with qualifying groundwater from the bottom up. Completely filled bottles were sealed with Teflon-lined caps, labeled, and placed in dark ice filled coolers for shipment to the treatability lab by overnight courier.

Upon receipt, the treatability lab anaerobically blended and homogenized all groundwater collected from monitoring well PC-24-PS into a single sterile container, which was stored in the dark at 15°C until used to construct the slurried microcosm samples. Upon receipt, the treatability lab anaerobically blended and homogenized all groundwater collected from monitoring well PC-28-D into a single sterile contained, which was stored in the dark at 15°C until used to construct the slurried microcosm samples.

2.3 Spiking Groundwater Feedstocks

Groundwater feedstocks were spiked with target volatile organic compounds (VOCs) because in-house analysis of preliminary slurries manufactured from raw soil and groundwater feedstocks showed their microcosms would produce aqueous target VOC concentrations below relevant levels. Target initial aqueous VOC concentrations for meaningful testing of microcosms were determined based on observation of typical magnitude and distribution of VOC compounds in relevant site groundwater. In-house experimentation resulted in a recipe for spiking the groundwater feedstocks with target VOCs to achieve relevant initial aqueous concentrations of target VOCs in the mixed slurry microcosms when mixed with each soil type.

Table 1 compares the target initial aqueous concentrations in spiked microcosms with their actual initial concentrations after spiking as represented results on Day 0 of the Natural Control microcosms.

Table 1. Comparison of target initial aqueous concentrations in spiked microcosms with their actual initial concentrations.

Raw Groundwater Well ID		PC-24P S		PC-24P S		PC-28D	
Companion Soil Type		Peat		Silt		Sand & Gravel	
Constituent	Units	Target Initial Aqueous Concentration	Actual Initial Aqueous Concentration in Natural Control	Target Initial Aqueous Concentration	Actual Initial Aqueous Concentration in Natural Control	Target Initial Aqueous Concentration	Actual Initial Aqueous Concentration in Natural Control
Tetrachloroethylene	µg/L	125	58	125	119	250	202
Trichloroethylene	µg/L	200	127	200	195	250	240
Cis-1,2-Dichloroethylene	µg/L	1,250	1,227	1,250	1,073	2,000	2,180
Trans-1,2-Dichloroethene	µg/L	125	281	125	361	250	308
Vinyl Chloride	µg/L	325	528	325	366	500	349
1,1,1-Trichloroethane	µg/L	125	77	125	115	250	303
1,1-Dichloroethane	µg/L	125	147	125	129	250	315
Chloroethane	µg/L	125	152	125	112	250	245
Total Detected Targets	µg/L	2,400	2,597	2,400	2,470	4,000	4,142

2.4 Reagent Feedstocks

Reagents were procured directly from their respective suppliers and stored under nitrogen blanket until used to manufacture the microcosms. Descriptions of the reagent are as follows:

- Fermentable Carbon Substrate (contains no reducing agent) capable of enhancing microbial reductive dechlorination:
  - ABC supplied by Redox Tech:
    - Moniker for Anaerobic BioChem
    - Blend of soluble lactic acid, ethyl lactate, and long lasting C14 to C18 fatty acids
    - Phosphate (essential nutrient and pH buffer)
    - (Redox Tech, LLC, 2012).
- Zero valent iron (ZVI) in the form of ZVI powder capable of enhancing abiotic chemical reduction and/or microbially-mediated chemical reduction:
  - Peerless 50D (variable meso-sized; 70% 45 to 250 µm, 30% <45 µm); (Peerlessmetal.com).
  - Hepure HCA 325 (uniform micro-sized; 90% <45 µm); (Hepure Metals, 2009).
- Blended Reagent (contains a mixture of fermentable carbon substrate and ZVI reducing agent) capable of enhancing synergistic microbial dechlorination, microbially-mediated dechlorination, and abiotic dechlorination:
  - ABC+ supplied by Redox Tech:
    - Moniker for Anaerobic BioChem PLUS ZVI
    - Blend of soluble lactic acid, ethyl lactate, long lasting C14 to C18 fatty acids, and ZVI powder (consistent with Peerless 50D)
    - Phosphate (essential nutrient and pH buffer)
    - (Redox Tech, LLC, 2012).
  - EHC F supplied by PeroxyChem (formerly FMC, formerly Adventus):
    - Moniker for Eh Compound Fine-grind
    - Blend of cellulose/hemicellulose, ferrous sulfate, and ZVI (consistent with Hepure HCA-325)
    - (PeroxyChem, 2014).

2.5 Microbial Inoculate Feedstocks

Some of the microcosms were inoculated with dechlorinating bacteria cultures after six months of testing. Inoculates were obtained directly from the treatability lab, which is one of the main suppliers of commercially available dechlorinating bacteria consortia. While these bacterial consortia contain a variety of microorganisms, including those that produce vitamin B12-like corrinoid cofactors of the type known to be requisite for *Dehalolcoccoides* spp. to dechlorinate chloroethenes to completion, the primary microbial targets were:

- *Dehalococcoides* spp. (DHC): supplied as Shaw Dechlorinating Consortium (SDC-9)
- *Dehalobacter* spp. (DHB): supplied by CB&I (formerly Shaw) commercial availability pending.

2.6 Construction of Microcosms

Five (5) replicate mixed slurry microcosms were anaerobically constructed in sterile 160-milliliter (mL) serum bottles for each of the three soil types and their respective designated spiked groundwater for each of the five test reagents. Seven (7) replicate un-amended natural control mixed slurry microcosms were anaerobically constructed in 160-mL serum bottles for each of the three soil types and their respective designated spiked groundwater. Five (5) replicate un-amended sterilized ("killed") control mixed slurry microcosms were anaerobically constructed in 160-mL serum bottles for each of the three soil types and their respective designated spiked groundwater, where the killed control microcosms were serialized by triplicate autoclaving. The general microcosm recipes were:

- 10 grams (g) of qualifying soil (drained but moist)
- 130 mL of qualifying groundwater
- Reagent (0.3 grams of fermentable carbon substrate and/or 0.2 grams ZVI; except controls)
- Sterile glass beads as filler where needed to achieve total volume of 160 mL
- Minute headspace is nitrogen gas
- Dechlorinating bacteria inoculate after six months in ABC, ABC+, and EHC-F microcosms
- Repeat reagent dosing after six months (except Peerless 50D in peat microcosms and Hepure HCA-325 in peat microcosms).

The microcosm construction site and typical finished microcosms are shown in Figure 3. Summaries of microcosm construction for each soil type are presented in Table 2, Table 3, and Table 4.

Figure 3. Typical microcosm construction site and finished microcosms.

Table 2. Peat microcosm construction recipes.

Peat Microcosms Construction Summary										
Date	**September 19, 2012**						**March 20, 2013**			
Elapsed Time (Days)	**0**						**183**			
Microcosm ID	**Peat (grams)**	**VOC-Spiked Groundwater (mL)**	**Regent (grams)**		**Microbial Inoculate (cells/mL of slurry)**		**Reagent (grams)**		**Microbial Inoculate (cells/mL of slurry)**	
			Fermentable Substrate	**Zero Valent Iron**	**DHC[2] (SDC-9)**	**DHC[2] (SDC-9)**	**Fermentable Substrate**	**Zero Valent Iron**	**DHC[2] (SDC-9)**	**DHC[2] (SDC-9)**
Natural Control	**10**	**130**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Killed Control	**10**	**130**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
ABC	**10**	**130**	**0.3**	**0**	**0**	**0**	**0.3**	**0**	**28,000**	**18,000**
Peerless 50D	**10**	**130**	**0**	**0.2**	**0**	**0**	**0**	**0**	**0**	**0**
ABC +	**10**	**130**	**0.3**	**0.2**	**0**	**0**	**0.3**	**0.2**	**28,000**	**18,000**
Hepure HCA-325	**10**	**130**	**0**	**0.2**	**0**	**0**	**0**	**0**	**0**	**0**
EHC-F	**10**	**130**	**0.3**	**0.2**	**0**	**0**	**0.3**	**0.2**	**28,000**	**18,000**

Table 3. Silt microcosm construction recipes.

Silt Microcosms Construction Summary										
Date	**September 26, 2012**						**March 21, 2013**			
Elapsed Time (Days)	**0**						**176**			
Microcosm ID	**Silt (grams)**	**VOC-Spiked Groundwater (mL)**	**Regent (grams)**		**Microbial Inoculate (cells/mL of slurry)**		**Reagent (grams)**		**Microbial Inoculate (cells/mL of slurry)**	
			Fermentable Substrate	**Zero Valent Iron**	**DHC[2] (SDC-9)**	**DHC[2] (SDC-9)**	**Fermentable Substrate**	**Zero Valent Iron**	**DHC[2] (SDC-9)**	**DHC[2] (SDC-9)**
Natural Control	**10**	**130**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Killed Control	**10**	**130**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
ABC	**10**	**130**	**0.3**	**0**	**0**	**0**	**0.3**	**0**	**28,000**	**18,000**
Peerless 50D	**10**	**130**	**0**	**0.2**	**0**	**0**	**0**	**0.2**	**0**	**0**
ABC +	**10**	**130**	**0.3**	**0.2**	**0**	**0**	**0.3**	**0.2**	**28,000**	**18,000**
Hepure HCA-325	**10**	**130**	**0**	**0.2**	**0**	**0**	**0**	**0.2**	**0**	**0**
EHC-F	**10**	**130**	**0.3**	**0.2**	**0**	**0**	**0.3**	**0.2**	**28,000**	**18,000**

Table 4. Sand & Gravel microcosm construction recipes.

Sand & Gravel Microcosms Construction Summary										
Date	**September 12, 2012**						**March 19, 2013**			
Elapsed Time (Days)	**0**						**188**			
Microcosm ID	**Sand & Gravel (grams)**	**VOC-Spiked Groundwater (mL)**	**Regent (grams)**		**Microbial Inoculate (cells/mL of slurry)**		**Reagent (grams)**		**Microbial Inoculate (cells/mL of slurry)**	
			Fermentable Substrate	**Zero Valent Iron**	**DHC[2] (SDC-9)**	**DHC[2] (SDC-9)**	**Fermentable Substrate**	**Zero Valent Iron**	**DHC[2] (SDC-9)**	**DHC[2] (SDC-9)**
Natural Control	**10**	**130**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Killed Control	**10**	**130**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
ABC	**10**	**130**	**0.3**	**0**	**0**	**0**	**0.3**	**0**	**28,000**	**18,000**
Peerless 50D	**10**	**130**	**0**	**0.2**	**0**	**0**	**0**	**0.2**	**0**	**0**
ABC +	**10**	**130**	**0.3**	**0.2**	**0**	**0**	**0.3**	**0.2**	**28,000**	**18,000**
Hepure HCA-325	**10**	**130**	**0**	**0.2**	**0**	**0**	**0**	**0.2**	**0**	**0**
EHC-F	**10**	**130**	**0.3**	**0.2**	**0**	**0**	**0.3**	**0.2**	**28,000**	**18,000**

3. RESULTS

The analytical schedule is shown in Table 5.

Table 5. Microcosm analytical schedule.

Analytical Chronology

Microcosm Soil Type	Date	Δtime (days)	Target VOCs[1]	Dissolved Gasses	Geochemistry											Carbon Substrate		Dechlorinating Bacteria[3] (or Their Enzyme Gene Expressions)					1,4-Dioxane
					D.O.	pH	ORP	Spec. Cond.	Temp-erature	Cl^-	o-PO_4	NO_3^-	NO_2^{2-}	Fe^{2+}	SO_4^{2-}	TOC	Volatile Fatty Acids[2]	*Dehalo-coccoides* spp.	tceA Reductase	BAV1 VC Reductase	Vinyl Chloride Reductase	*Dehalo-bacter* spp.	
Peat	9/19/2012	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	10/2/2012	14	X	X	X	X	X	X	X														
	11/5/2012	48	X	X	X	X	X	X	X	X	X	X	X	X	X		X						
	12/20/2012	93	X	X		X	X	X	X	X	X	X	X	X	X	X	X						
	1/23/2013	127	X	X		X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	
	3/20/2013	183	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
	3/20/2013	183	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				X	
	5/15/2013	239	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
	8/14/2013	330	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Silt	9/26/2012	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	10/11/2012	15	X	X	X	X	X	X	X														
	11/8/2012	43	X	X	X	X	X	X	X	X	X	X	X	X	X		X						
	12/27/2012	92	X	X		X	X	X	X	X	X	X	X	X	X	X	X						
	2/7/2013	134	X	X		X	X	X	X	X	X	X	X	X	X		X						
	3/21/2013	176	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
	3/21/2013	176	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				X	
	5/16/2013	232	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
	8/22/2013	330	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Sand & Gravel	9/12/2012	0	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
	9/27/2012	15	X	X	X	X	X	X	X														
	10/25/2012	43	X	X	X	X	X	X	X	X	X	X	X	X	X		X						
	12/13/2012	92	X	X		X	X	X	X	X	X	X	X	X	X	X	X						
	1/24/2013	134	X	X		X	X	X	X	X	X	X	X	X	X		X						
	3/19/2013	188	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
	3/19/2013	188	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				X	
	5/14/2013	244	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X						
	8/8/2013	330	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[1] Ethene, Ethane, Methane, Propane, Acetylene

[2] Lactic, Acetic, Propionic, Formic, Butyric, Pyruvic, Valeric

[3] Microbial CENSUS analyzed in all Natural Control microcosms on or about Day-0, and then only in Peat microcosms after 4.5 months of testing, and then only in ABC, ABC+, and EHC-F microcosms after about 5 months of bioaugmentation. DHC and DHBt densities upon bioaugmentation of the ABC, ABC+, and EHC-F slurries at the nominal 6-month point reflect in-house qPCR results from the treatability lab.

3.1 Destruction of Total Chloroethenes

The net loss of aqueous chloroethenes is shown in Figure 3.

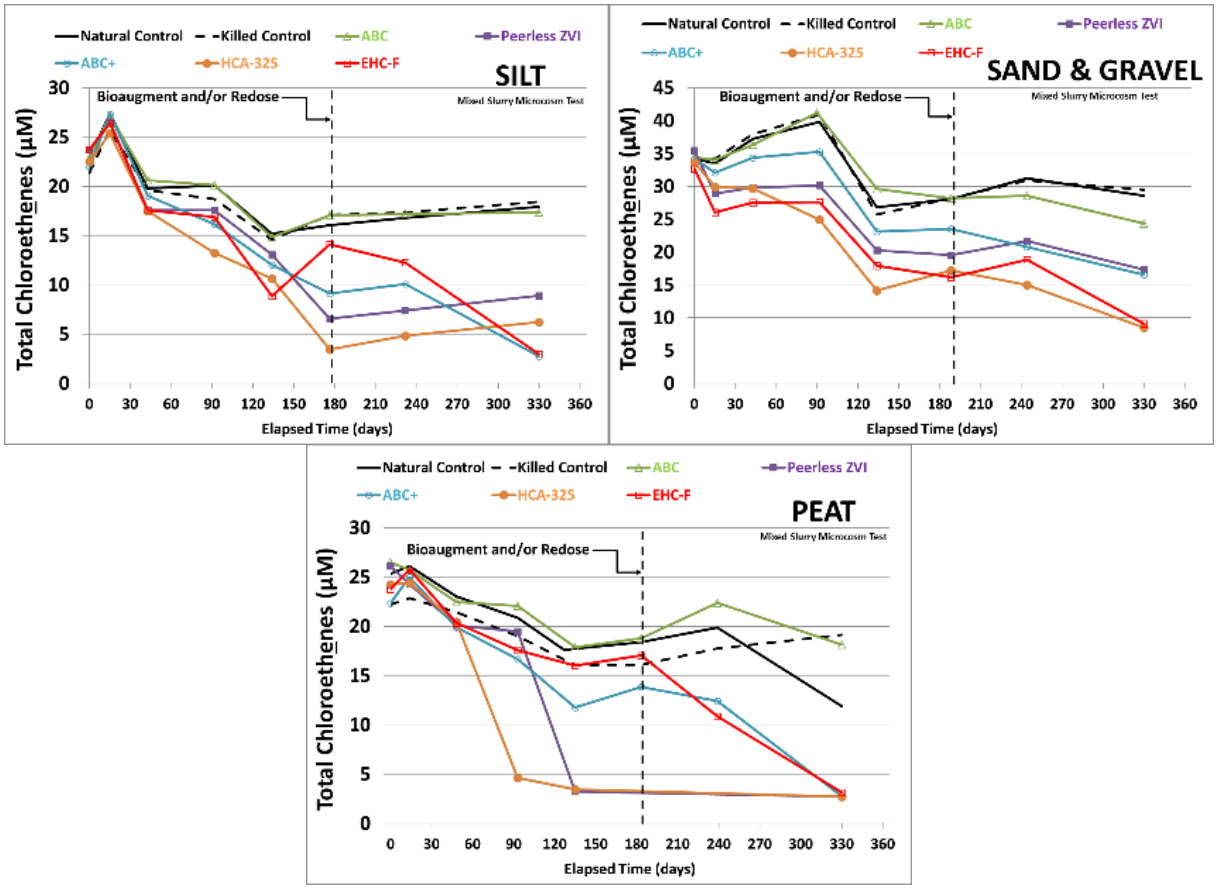

Figure 4. Total chloroethenes behavior as a function of test duration.

In general, degradation of chloroethenes was not observed in the microcosms amended with fermentable substrate only (ABC). Degradation of chloroethenes was observed in the ZVI and blended reagents (ZVI and fermentable substrate) in all soil types, with the most significant changes observed in the blended reagents microcosms after bioaugmentation.

3.2 Fermentable Carbon Reagent ABC

Figure 5 shows chloroethenes dechlorination behavior inspired by fermentable carbon substrate reagent ABC. Figure 6 shows changes in aqueous ORP; the green line represents conditions inspired by reagent ABC. Figure 7 shows changes in pH behavior; the green line represents conditions inspired by reagent ABC.

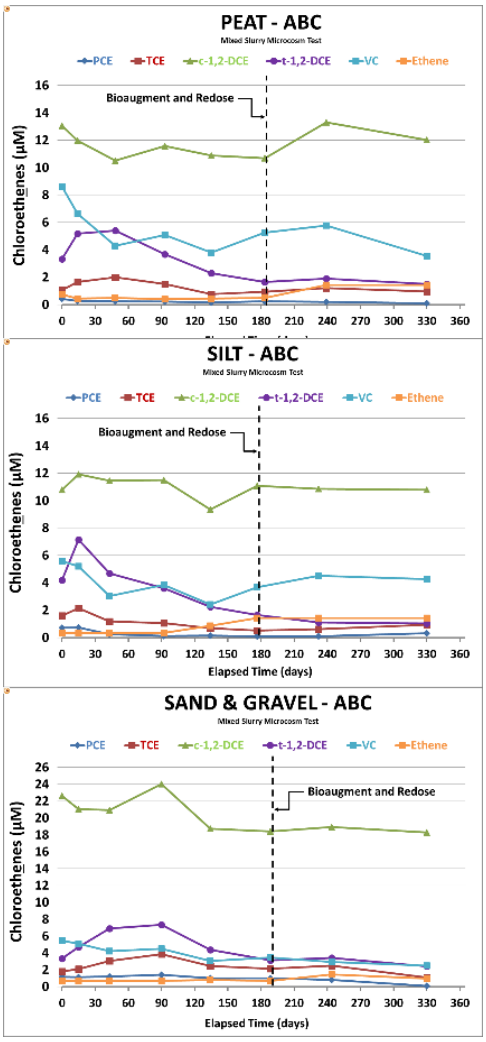

Figure 5. Chloroethenes dechlorination behavior with fermentable carbon substrate reagent ABC.

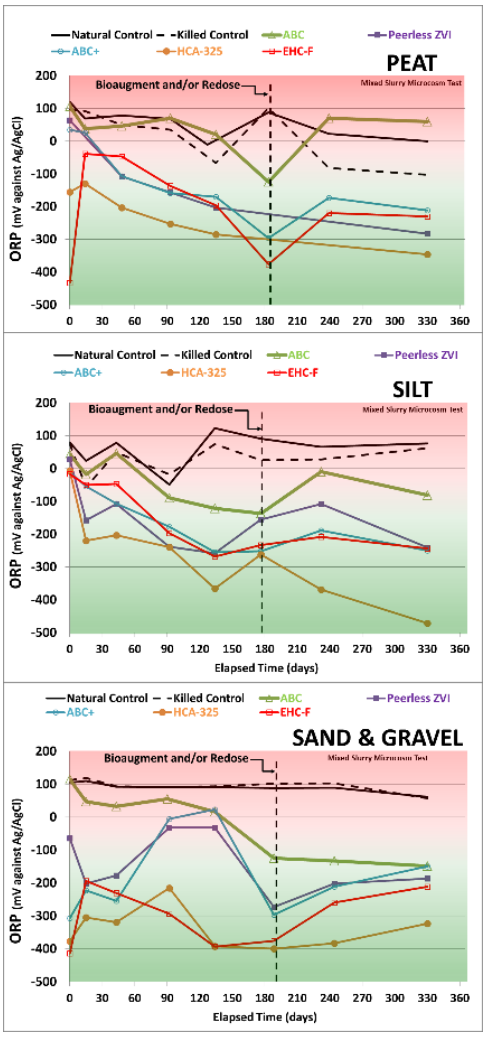

Figure 6. ORP behavior as a function of test duration. The green line represents the behavior inspired by fermentable carbon substrate ABC.

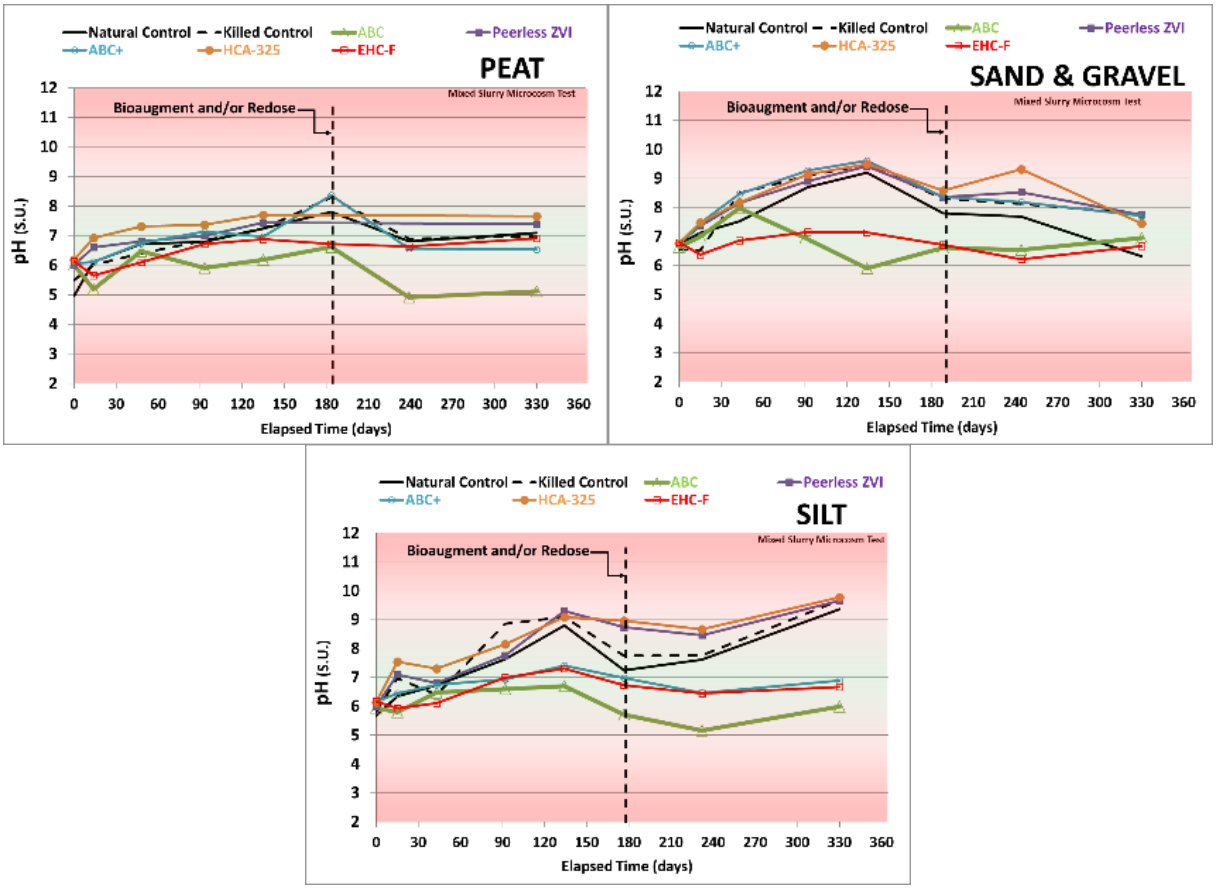

Figure 7. pH behavior as a function of test duration. The green line represents the behavior inspired by fermentable carbon substrate ABC.

Dechlorination of the chloroethenes suite of target compounds was not effectively enhanced by the fermentable carbon substrate reagent ABC in any of the soil types. As shown in the Figure 5, concentrations of both parent and degradation daughter compounds remained generally constant during the ABC test.

Failure of fermentable carbon substrate ABC to effectively enhance dechlorination in any of the microcosms under test conditions may be attributable to its inability to induce sufficient reducing conditions at which reductive dechlorination can occur at meaningful rates (i.e. ORP less than -200 mV). As shown in Figure 6, the ABC reagent microcosms remained above the -200 mV threshold throughout the entire test.

Failure of fermentable carbon substrate ABC to effectively enhance dechlorination in any of the microcosms under test conditions may also be attributable to its proclivity to drive pH into the suboptimum range for dechlorinating bacteria (i.e. pH <6 s.u.), as shown in Figure 7. ABC inspired the Peat and Silt microcosms to achieve a pH condition of close to 5 s.u., which is inhibitory to the activity of essential *Dehalococcoides* (DHC) bacteria. The data are inconclusive regarding prospect for future pH to rebound back up to agreeable levels after the initial ABC-inspired depletion.

3.3 Zero Valent Iron (ZVI) Reagents (Peerless 50D and Hepure HCA-325)

Figure 8 shows chloroethenes dechlorination behavior inspired by ZVI reagents Peerless 50D and Hepure HCA-325.

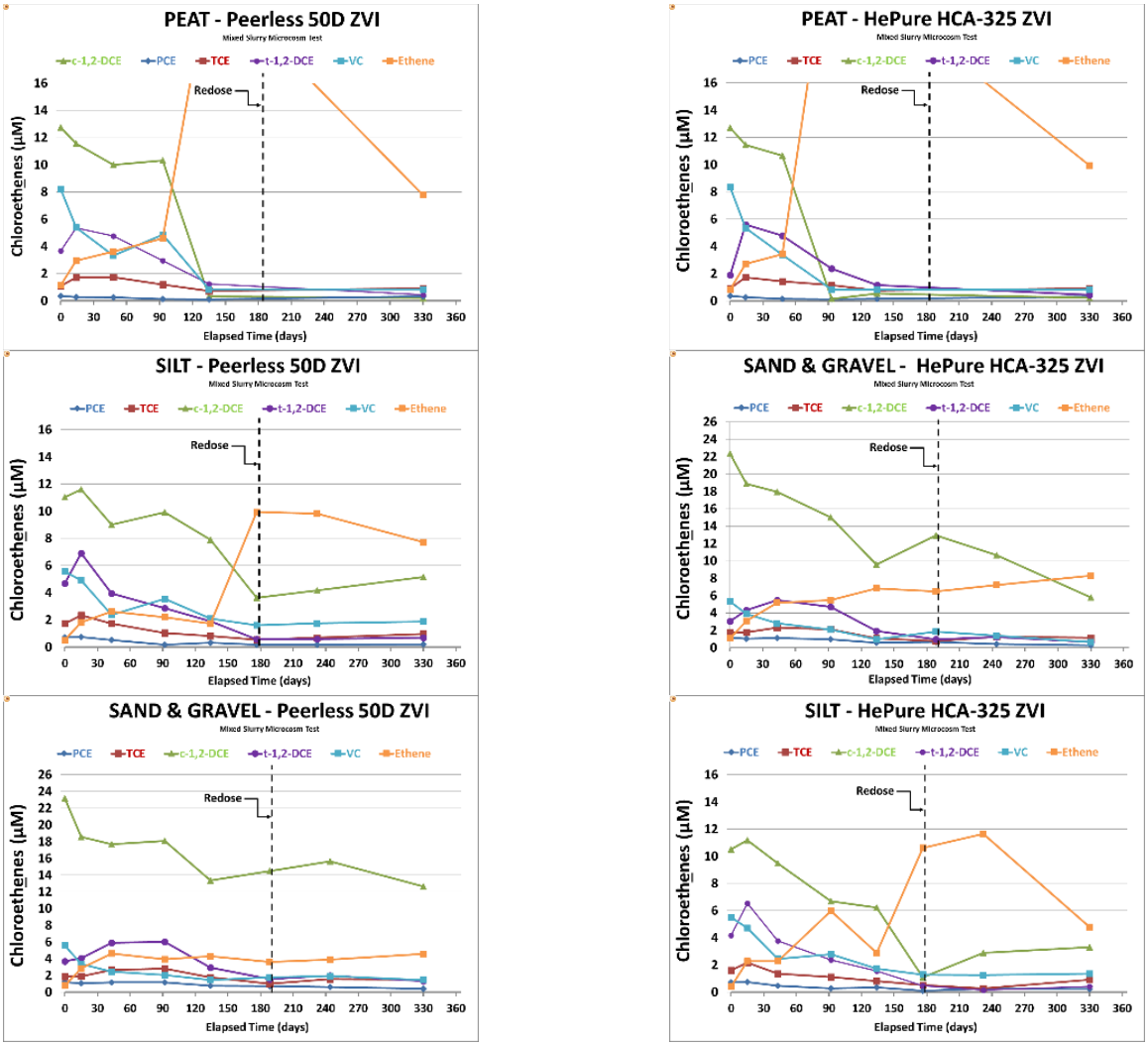

Figure 8. Chloroethenes dechlorination behavior with ZVI reagents.

The microcosm tests showed little differences in dechlorination performance between the two ZVI reagents tested, which indicates that overall dechlorination of chloroethenes is independent of ZVI particle size. The smaller-sized Hepure HCA-325 ZVI appeared promote more rapid onset of reactivity than the larger-sized Peerless 50D ZVI. It is reasonably expected that the smaller ZVI particles in Hepure HCA-325 provide higher reactivity and lower longevity than the larger particles in Peerless 50D. ZVI longevity and passivation as a function of ZVI particle size, or as a function of soil type, were inconclusive based solely on these data. Further, no benefit (additional concentrations declines) was garnered by redosing the microcosms with ZVI, which indicates that passivation of the ZVI had not occurred and degradation was limited by other processes.

Figure 9 shows changes in aqueous ORP; the purple line represents the behavior inspired by Peerless 50D ZVI powder; the orange line represents behavior inspired by Hepure HCA-325 ZVI powder.

Figure 10 shows changes in pH behavior; the purple line represents the behavior inspired by Peerless 50D ZVI powder; the orange line represents behavior inspired by Hepure HCA-325 ZVI powder.

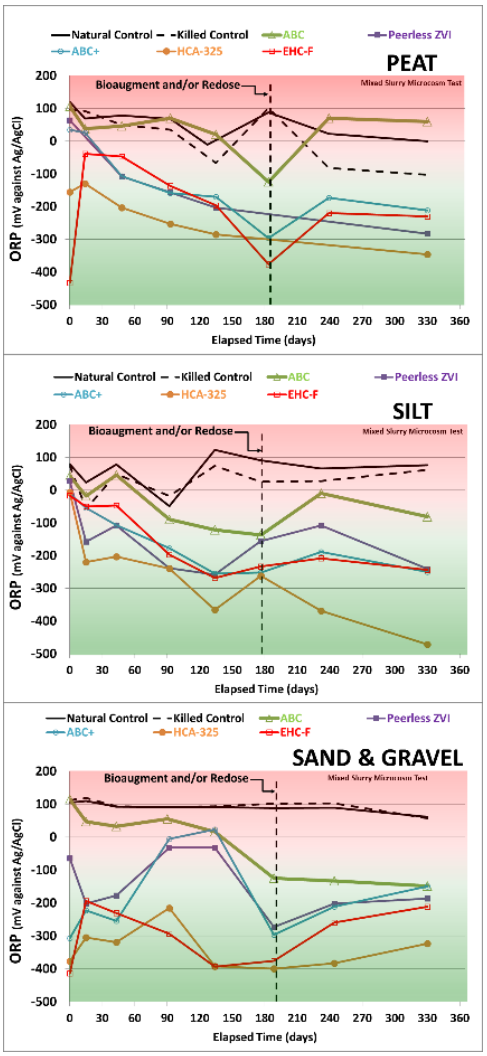

Figure 9. ORP behavior as a function of test duration. The purple line represents the behavior inspired by Peerless 50D ZVI powder. The orange line represents the behavior inspired by Hepure HCA-325 ZVI powder.

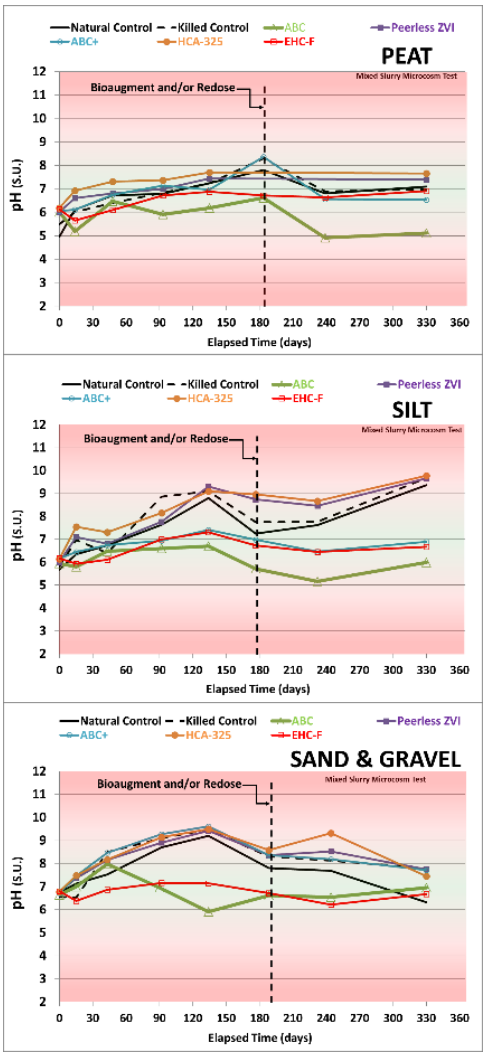

Figure 10. pH behavior as a function of test duration. The purple line represents the behavior inspired by Peerless 50D ZVI powder. The orange line represents the behavior inspired by Hepure HCA-325 ZVI powder.

Change in ORP induced by the ZVI reagents were reasonably parallel in all microcosms. The smaller-sized Hepure HCA-325 ZVI lowered ORPs significantly more than the larger-sized Peerless 50D ZVI. The smaller-sized Hepure HCA-325 ZVI appears to be able to promote reducing conditions below -300 mV in all soil types.

ZVI reagents increased pH in all microcosms; reaching or approaching high pH inhibitory levels greater than 9 s.u. permanently in Silt microcosms and temporarily in Sand and Gravel microcosms. ZVI in peat microcosms caused pH to rise and remain at circumneutral levels slightly greater than 7 s.u. However, the impact of ZVI on pH is somewhat inconclusive because similar pH behaviors were observed in both natural and killed controls.

3.4 Blended (Fermentable Carbon Substrate + ZVI) Reagents (ABC+ and EHC-F)

Figure 11 shows chloroethenes dechlorination behavior inspired by blended reagents ABC+ and EHC-F

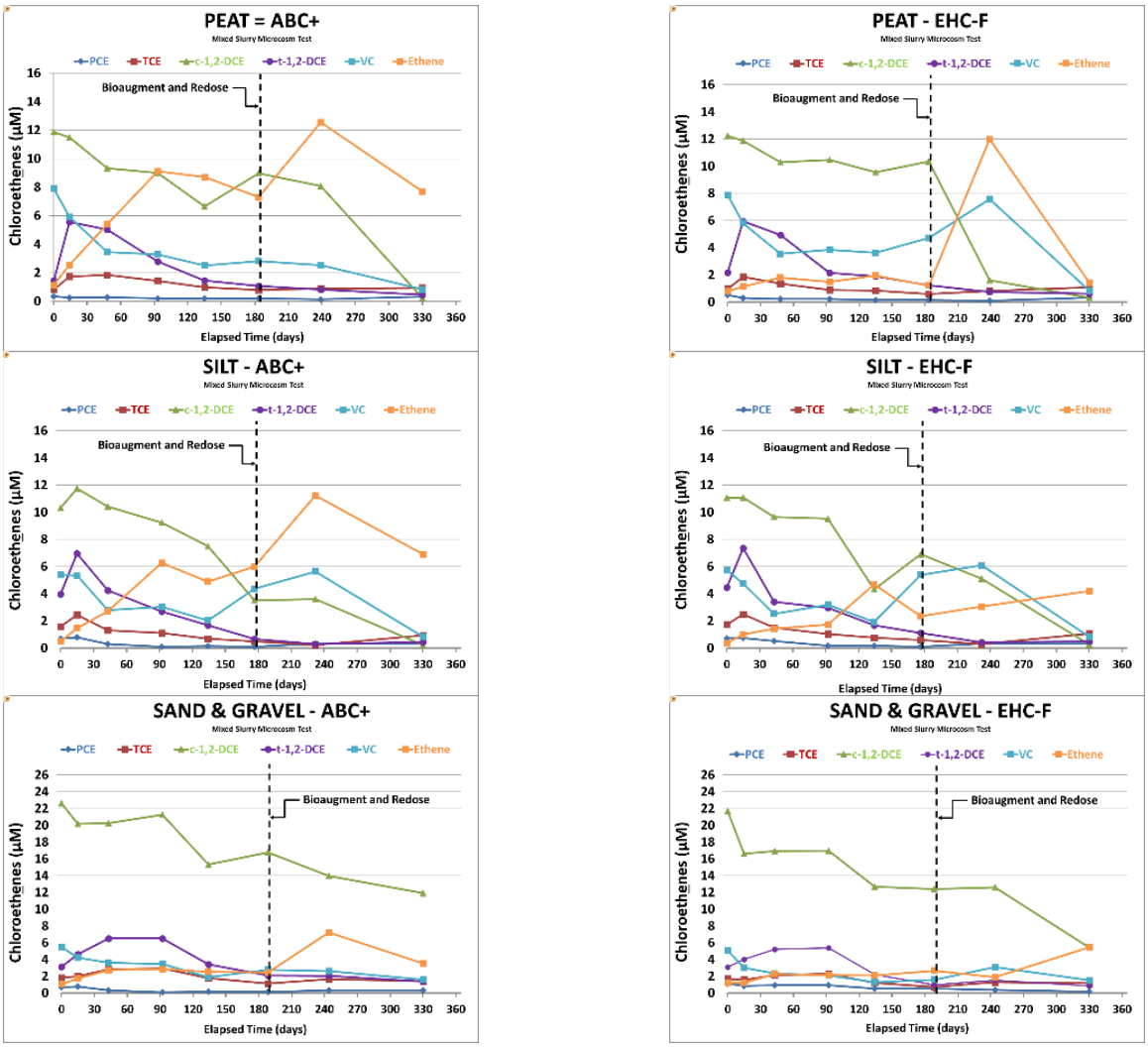

Figure 11. Chloroethenes dechlorination behavior with blended reagents (fermentable carbon substrate plus ZVI).

Relatively low dechlorination rates (prior to bioaugmentation) were observed in the first 180 days of the test for the Peat and Sand and Gravel microcosms, which is plausibly attributable mostly to ZVI-inspired abiotic mechanisms. Higher pre-bioaugmented dechlorination rates observed in the Silt microcosms suggests that some dechlorination may be attributable to biodegradation.

Figure 12 shows changes in aqueous ORP; the blue line represents the behavior inspired by ABC+; the red line represents the behavior inspired by EHC-F. Figure 13 shows changes in pH behavior; the blue line represents the behavior inspired by ABC+; the red line represents the behavior inspired by EHC-F.

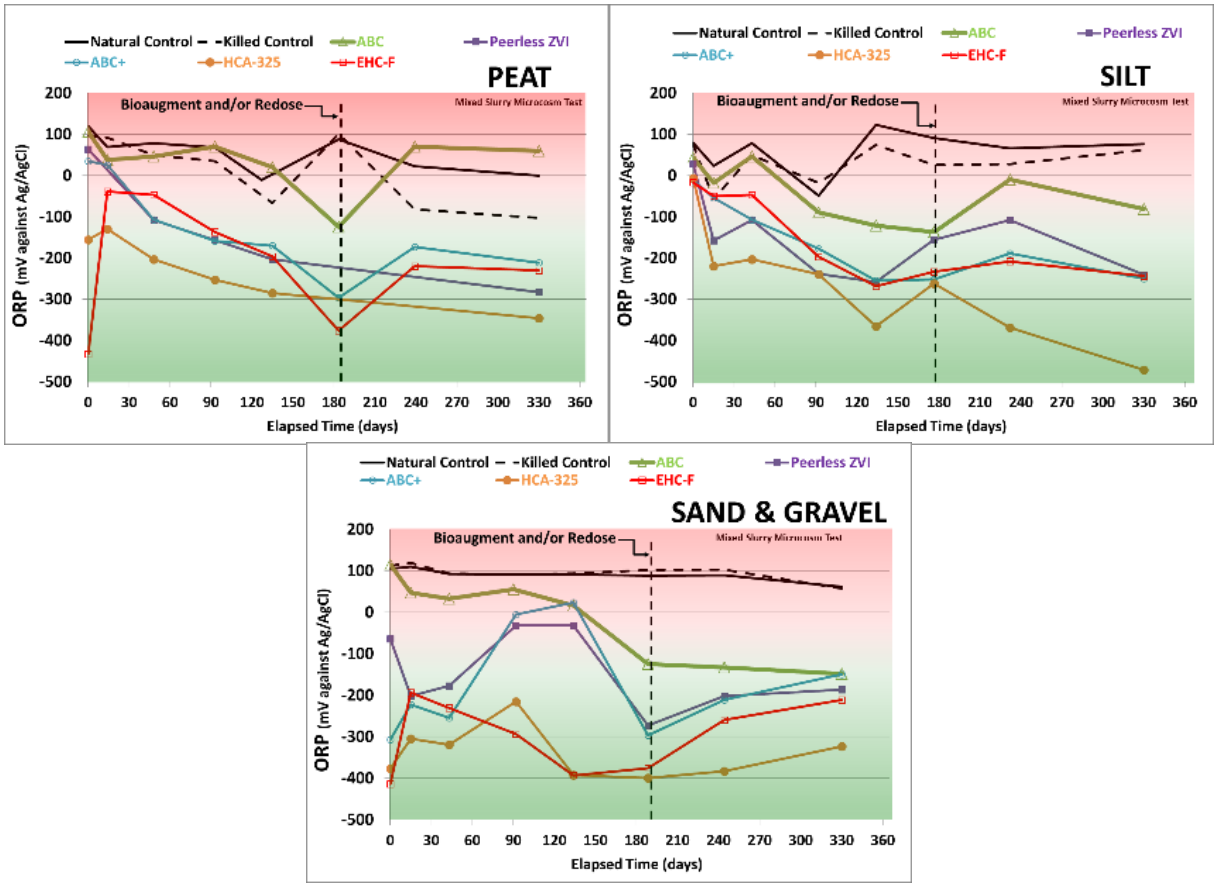

Figure 12. ORP behavior as a function of test duration. The blue line represents the behavior inspired by ABC+. The red line represents the behavior inspired by EHC-F.

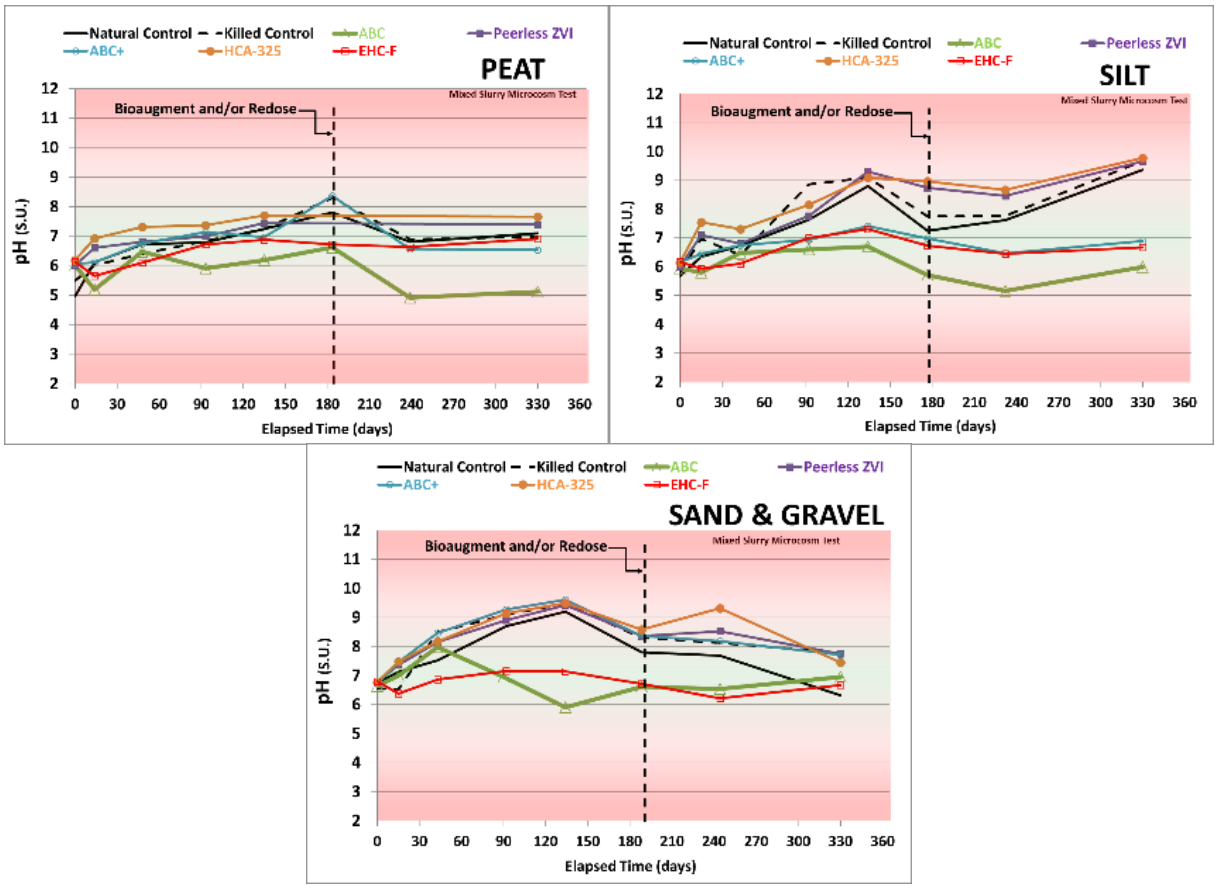

Figure 13. pH behavior as a function of test duration. The blue line represents the behavior inspired by ABC+. The red line represents the behavior inspired by EHC-F.

4. DISCUSSION/CONCLUSIONS

The results show that pH, in a poorly-buffered environment, can be induced to degradation rate-limiting or rate-inhibiting values under conditions of the test:

- pH too low in the case of amending only with fermentable carbon substrate
- pH too high in the case of amending with only ZVI.

However, amending fermentable carbon substrate mixed with ZVI attenuated pH migration into degradation rate-inhibiting territories, both low and high.

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BIOREMEDIATION OF CHLOROETHENES IN GROUNDWATER BY MIXING ANAEROBIC BACTERIA DIRECTLY INTO FERMENTABLE SUBSTRATE

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ABSTRACT

Tetrachloroethene (PCE) degradation in groundwater was stimulated to completion when an anaerobic dechlorinating bacteria culture was mixed directly into the fermentable carbon substrate injectate prior to direct injection of the reagent mixture into the subsurface. This manuscript presents the two-year performance results of an on-going bioremediation pilot test in PCE-impacted groundwater.

It is evidenced that cultured anaerobic dechlorinating bacteria survived mixing into a concentrated fermentable carbon substrate solution prior to injecting the substrate-bacteria mixture into the targeted subsurface. qPCR testing indicates that dechlorinating bacteria with genes coding for enzymes known to be responsible for anaerobic reductive dechlorination of PCE and its degradation daughter products to non-toxic ethene continued to thrive at meaningful densities in the subsurface after two years. Results evidence change in activity of different strains of *Dehalococcoides* bacteria are concomitant with changes in mole fractions of the chlorinated compounds. These positive results contrast with the popular opinion that bioaugmentation should be performed separately and after delivery of biostimulants.

Keywords: bioaugmentation, biostimulation, *Dehalococcoides*, tetrachloroethene, qPCR, injection

1. INTRODUCTION

A bioremediation pilot test was conducted at an industrial facility in Charlotte, North Carolina, United States, to assess efficacy of enhancing in situ dechlorination of tetrachloroethene (PCE) and its sequential degradation products trichloroethene (TCE), isomers of dichloroethene (DCE), and vinyl chloride (VC) using an injectate manufactured by inoculating anaerobic dechlorinating bacteria directly into an anoxic aqueous solution of fermentable carbon substrate. The injectate was injected into a PCE-impacted unconfined sandy silt aquifer with a hydraulic conductivity of approximately 0.1 feet per day (ft/day) [3.5×10^{-5} cm/sec] using direct-push methods.

Conventional remediation industry practice (AFCEE, 2004) is to conduct biostimulation prior to bioaugmentation as opposed to mixing bacteria cultures directly with biostimulants and injecting as a mixture. Rationale driving decisions to biostimulate prior to bioaugmentation fall generally into the following two categories:

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1. Prospect that carbon substrate solution will kill or inactivate the bacteria if the bacteria are mixed into the injectate.
2. Prospect that native aquifer conditions, especially pH and dissolved oxygen (Vainberg *et al.* 2006; USEPA 2010; Gentry *et al.* 2004), will be inhospitable to inoculated bacteria until preconditioned by biostimulants.

This research team hypothesized that a single injectate manufactured by mixing anaerobic dechlorinating bacteria culture directly into anoxic aqueous fermentable carbon substrate solutions could be used successfully. The approach used native anoxic groundwater for injectate makeup water, and avoided oxygen, ultraviolet light, and high temperature during the injectate manufacturing process. Testing showed that inoculated bacteria survived injectate preparation, and the inoculated bacteria thrived in the aquifer for more than 24 months.

2. MATERIALS AND PROCEDURES

A total of 1,500 gallons of injectate was manufactured for this test. The injectate consisted of carbon substrate, native anoxic groundwater pumped from on-site groundwater monitoring wells, and an anaerobic bacteria culture.

The makeup groundwater was pumped to holding tanks (330-gallon plastic totes) shielded from the sun with tarps to protect native bacteria from ultraviolet radiation poisoning. Anoxic (or microoxic) conditions were maintained by providing a nitrogen headspace in the holding tanks. Dixie Crystals® table sugar and Fleischmann's® yeast were added to the makeup water at 0.8 and 0.008 weight percent, respectively, to further assure maintenance of anoxic makeup water.

The fermentable carbon substrate was Reodox Tech's Anaerobic BioChem (ABC) product; a mixture of lactates, fatty acids, and phosphate buffer. The bacteria feedstock used for this test was the SDC-9^{TM} culture from Shaw Environmental, Inc. consisting of *Dehalococcoides* spp. (DHC) with densities greater than $1x10^{13}$ *DHC* cells per milliliter (cells/mL).

A 20 weight percent solution of ABC fermentable carbon substrate was first manufactured by mixing 281 gallons (about 2,700 pounds) of neat ABC reagent into 1,219 gallons of anoxic makeup groundwater in a light-proof, sun-shaded, bladder tank. Five gallons (19 liters) of SDC-9 bacteria was then blended in while recirculating the solution through the tank from one end to the other with a centrifugal pump.

Figure 1 illustrates the setup..

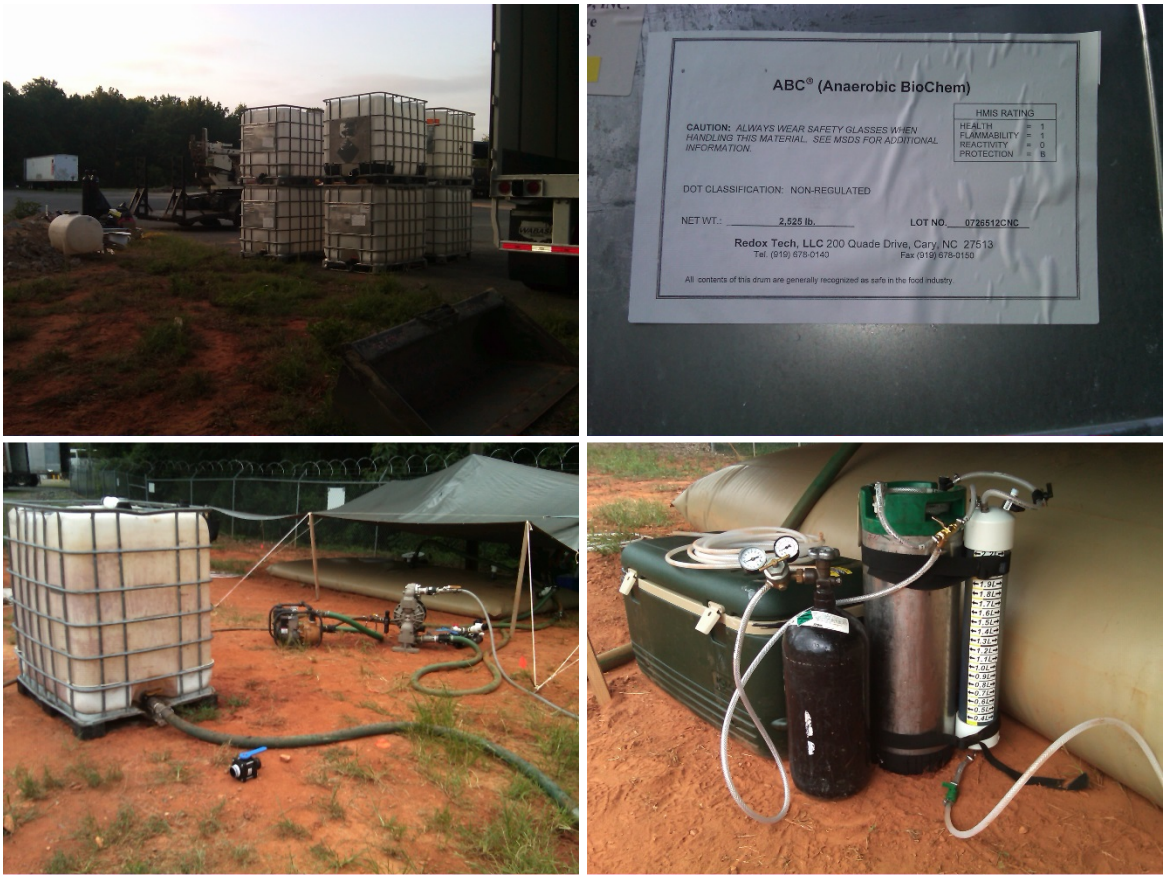

Figure 1. Injectate manufacturing setup.

Prior to injection, the inoculated injectate was sampled through Bio-Flo filter samplers and tested by Microbial Insights, Inc. for *Dehalococcoides* spp. and *Delahobacter* spp. using the quantitative polymerase chain reaction (qPCR) CENSUS analysis. The injectate was also analyzed for the following functional gene expressions of DHC:

- TCE Reductase (tceA) – expresses the enzyme responsible for dechlorination of TCE to cis-DCE
- Vinyl Chloride Reductase (vcrA) – expresses the enzyme responsible for dechlorination of cis-DCE to VC and for dechlorination of VC to ethene
- BAV1 Vinyl Chloride Reductase (bvcA) – expresses the enzyme responsible for dechlorination of VC to ethane.

The injectate was also characterized for additional analytes and geochemical parameters including pH, dissolved oxygen (DO), oxidation-reduction potential (ORP), conductivity, volatile fatty acids, dissolved gases including ethene, ethane, and methane, and the target volatile organic compounds (VOCs).

Figure 2 is a photograph of the injectate sampling apparatus.

Figure 2. Injectate sampling apparatus.

The inoculated injectate was then injected into three direct-push points arranged center and ends of a quarter-disk circumference located 10 feet upgradient of groundwater monitoring well, AC-3. Five hundred (500) gallons of inoculated injectate was injected at each point at nominal five-foot intervals between 17.5 feet below ground surface (ft bgs) and 27.5 ft bgs. Total injectate volume was 1,500 gallons.

Figure 3 illustrates the injection apparatus.

Figure 3. Injection apparatus.

Groundwater at the monitoring well AC-3 was sampled using low-flow methodology for parameters and constituents that were analyzed and recorded for the injectate mixture. The monitoring well was sampled prior to injection and at various intervals over 24 months.

3. RESULTS

Table 1 shows results of baseline (pre-injection) groundwater quality in monitoring well AC-3 for selected analytes.

Table 1. Baseline (pre-injection) groundwater quality in monitoring well AC-3.

AC-3	PCE µg/L	TCE µg/L	cis-1,2-DCE µg/L	VC µg/L	Ethene µg/L	DHC Density cells/mL	Total Bacteria Density cells/mL	pH s.u.	ORP mV	DO mg/L	Temp °C
	6,100	1,200	3,300	150	12	1.1E1	6.42E2	6.5	+234	0.8	22

Table 2 presents results of inoculated injectate quality for selected analytes.

Table 2. Injectate quality.

Injectate	PCE µg/L	TCE µg/L	cis-1,2-DCE µg/L	VC µg/L	Ethene µg/L	DHC Density cells/mL	Total Bacteria Density cells/mL	pH s.u.	ORP mV	DO mg/L	Temp °C
	230	75	270	450	880	5.53E6	2.07E7	7.0	+79	0.7	26

Table 3 displays changes in AC-3 groundwater quality for selected analytes.

*Table 3.*Change in AC-3 groundwater quality.

Days Following Injection	PCE µg/L	TCE µg/L	cis-1,2-DCE µg/L	VC µg/L	Ethene µg/L	DHC Density cells/mL	Total Bacteria Density cells/mL	pH s.u.	ORP mV	DO mg/L	Temp °C
0	6,100	1,200	3,300	150	12	1.1E1	6.42E2	6.5	+234	0.8	22
163	510	67	7,100	510	110	2.07E4		6.3	-180	0.3	20
232	50	51	3,500	880	180	1.39E5		6.1	-119	0.4	17
321	40	60	1,500	490	340	4.08E4	5.06E5	5.9	-81	0.2	26
384	480	590	2,300	740	260	4.06E4	1.50E5	6.5	-124	0.5	23
576	190	62	810	300	77	1.30E5	4.78E6	5.1	-24	0.9	16
737	18	21	400	410	260	2.78E6	1.13E7	5.8	-130	0.9	30

Figure 4 compares changes in DHC density to changes in chloroethenes concentrations in AC-3 groundwater.

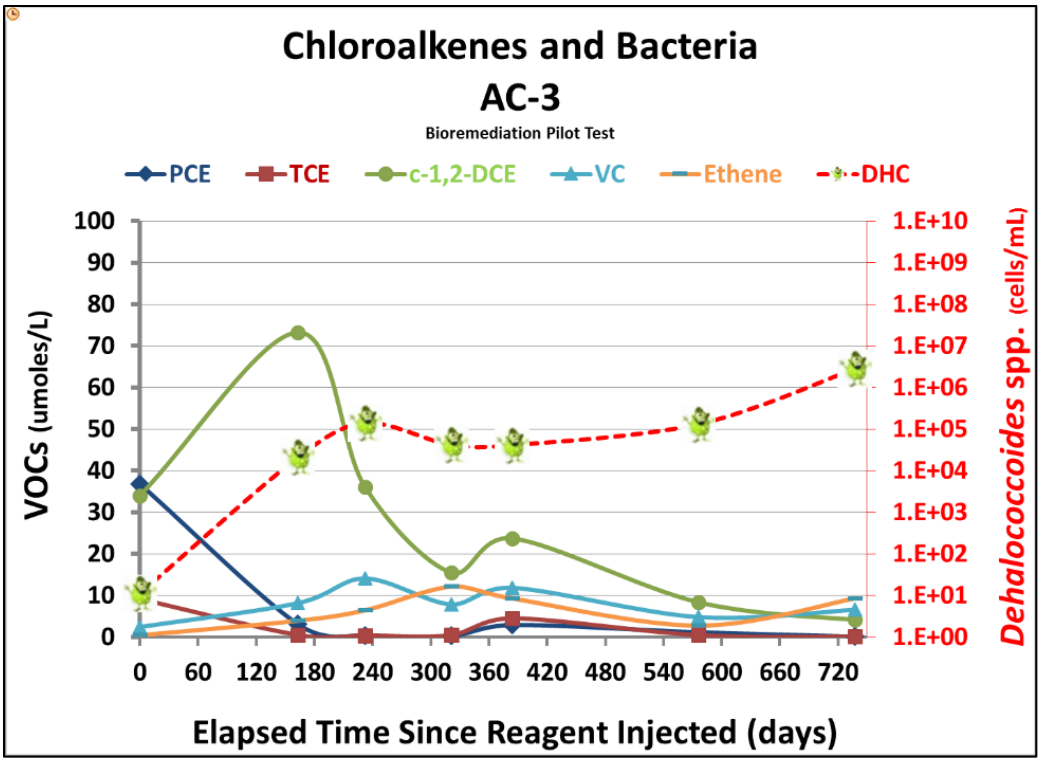

Figure 4. Change in *Dehalococcoides* spp. density and chloroethenes concentrations in AC-3 groundwater.

Genes that code for enzymes known to be capable of dechlorinating cis-1,2-DCE and/or VC to non-toxic ethene, bvcA and vcrA, are present in some, but not all DHC bacteria. Thus, the ratio of (bvcA+vcrA)/DHC is a metric that assists in the evaluation of the robustness of a DHC bacteria population with respect to prospect for microbial reductive dechlorination of chloroethenes all the way to non-toxic ethene. The closer this ratio is to unity, the greater the prospect for complete dechlorination to non-toxic ethene.

Table 4 shows the change in proportion of DHC bacteria that contain the genes coding for production of enzymes bvcA and vcrA in AC-3 groundwater, expressed as (bvcA+vcrA)/DHC.

Table 4. Change in proportion of DHC bacteria that contain the genes coding for production of enzymes bvcA and vcrA in AC-3 groundwater.

Days Following Injection	(bvcA+vcrA)/DHC
0	**0.05**
163	**0.09**
232	**0.07**
321	**0.07**
384	**1.41**
576	**0.26**
737	**0.42**

Figure 5 compares changes in chloroethenes mole fractions in AC-3 groundwater with changes in (bvcA+vcrA)/DHC ratio.

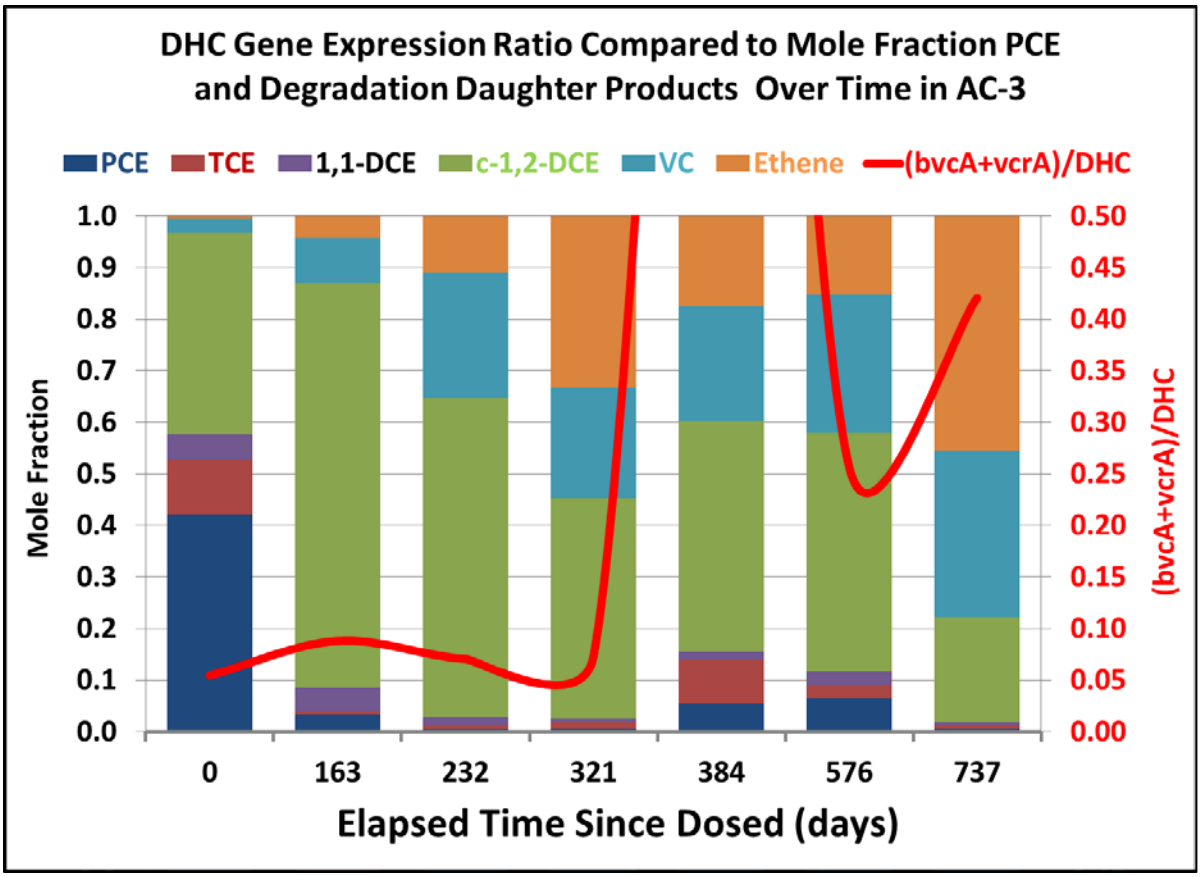

Figure 5. Changes in chloroethenes mole fractions in AC-3 groundwater compared to (bvcA+vcrA)/DHC ratios.

It is evident from Table 4 and Figure 5 that (bvcA+vcrA)/DHC on Day 384 may be biased high for some unknown reason. Figure 6 illustrates these changes in chloroethenes mole fractions in AC-3 groundwater with changes in (bvcA+vcrA)/DHC without the apparent bias of day-384 (bvcA+vcrA)/DHC.

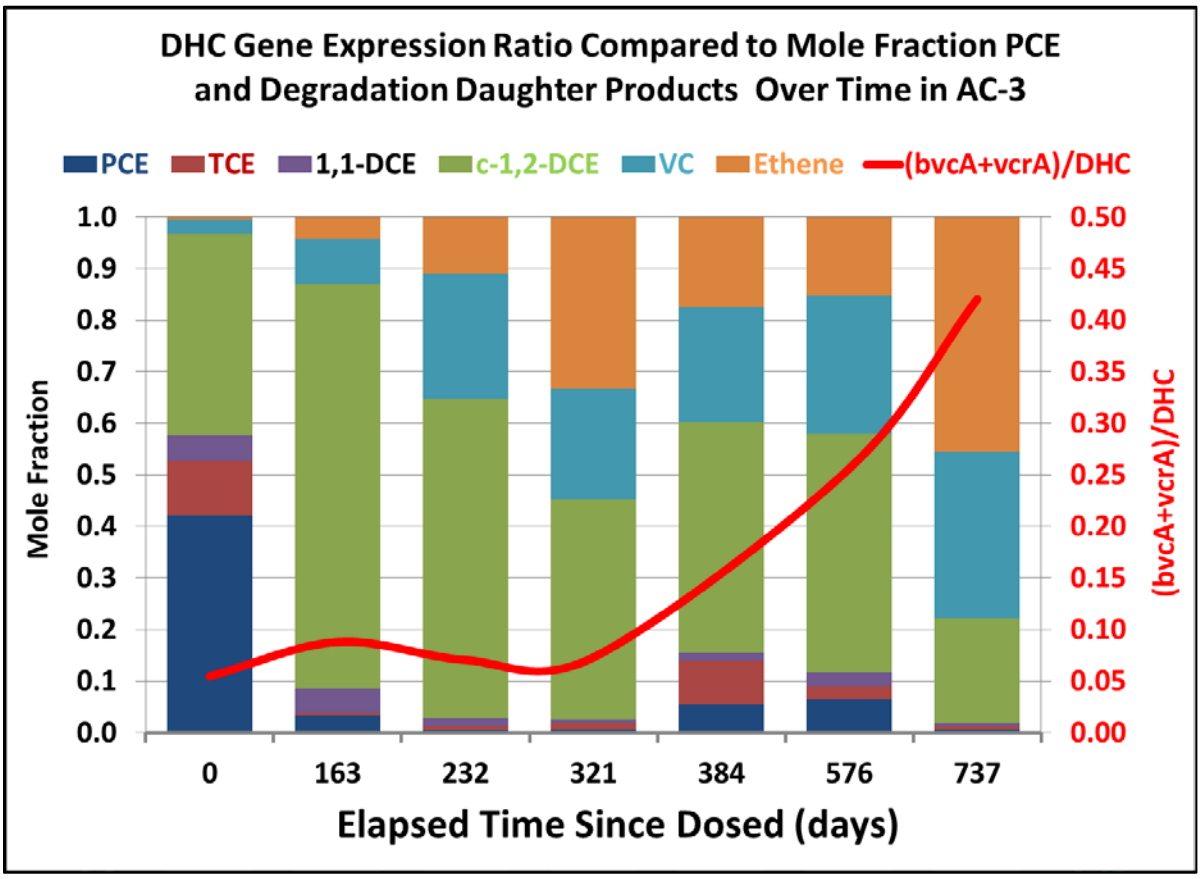

Figure 6. Changes in chloroethenes mole fractions in AC-3 groundwater compared with (bvcA+vcrA)/DHC ratios (neglecting apparent high-bias of Day-384 (bvcA+vcrA)/DHC value).

It is apparent from Figure 6 that the DHC bacteria population evolved in response to changes in prevalence of individual chloroethenes suite of compounds; (bvcA+vcrA)/DHC increased with increasing proportions of cis-1,2-DCE and/or VC.

4. DISCUSSION/CONCLUSIONS

Effective bioremediation of PCE-impacted groundwater was accomplished at this site through biostimulation-bioaugmentation techniques. Preconditioning of the aquifer with biostimulants in advance of bioaugmentation was found to be unnecessary. Bacteria mixed directly into the fermentable carbon substrate solution survived their transfer through the injectate solution and into the aquifer in quantities sufficient for effective remediation.

The data supports the research team's hypothesis that direct mixing of a dechlorinating bacteria culture with a fermentable carbon substrate prior to injection to the subsurface can be completed successfully as evidenced by meaningful degradation of PCE all the way to non-toxic ethene and survival of DHC bacteria at meaningful densities for at least two years after injection.

DHC cell density in the monitoring well AC-3 groundwater increased over the two-year monitoring period by five orders of magnitude. Robustness of the DHC community is further supported by the observation that increases in functional gene densities relative to total DHC density appear to correlate with increasing proportions of cis-1,2-DCE and VC compounds relative to the total chloroethenes concentration.

These data suggest the prospect of inoculating bacteria directly into a biostimulant solution prior to injection may be more efficacious than widely believed. Such successful applications can be expected to garner considerable cost savings compared to the more conventional approach of biostimulating and bioaugmenting in separate events.

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TOXICOLOGY AND ENVIRONMENTAL REGULATION OF 1,4-DIOXANE

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ABSTRACT

1,4-Dioxane is a clear liquid, miscible with water, that historically was used as a solvent stabilizer in chlorinated solvents, paint strippers, greases and waxes. Presently, it is found as a trace impurity in many household products such as detergents, shampoos, deodorants, toothpastes, and cosmetics, as well as in some food packaging and cooked foods. Due to its high environmental mobility, low volatility and recalcitrance to degradation, groundwater typically is the primary medium of interest at industrial facilities, both on-site and off-site. The extent of toxicity and associated risk from 1,4-dioxane is dependent on the exposure route (i.e., ingestion, inhalation, or dermal), the degree of exposure, and the duration of exposure. 1,4-Dioxane has the ability to cause liver and kidney injury by all routes of exposure in animal studies at sufficiently high dosage, but such effects are either not observed, or are greatly diminished at lesser exposure levels. Tumor studies historically have used very high dose exposures that have produced extensive liver toxicity, suggesting that cell damage and proliferation may be necessary for the formation of these tumors. However, the precise mechanism of action has not been determined. Available literature indicates that 1,4-dioxane is nongenotoxic or at worst weakly genotoxic in some test systems. As a result of improvements in analytical procedures and heightened awareness, 1,4-dioxane is detected with increasing frequency at cleanup sites around the country. This, coupled with a 10-fold increase (more restrictive) in the cancer slope factor (CSF) established by USEPA in 2010, has had a significant impact on recommended cleanup values, as well as cleanup methods and approaches. This paper provides a critical review of the toxicological data which has influenced recent state and federal regulatory values for 1,4-dioxane, including evaluation of risk-based cleanup values for 1,4-dioxane in drinking water, surface water, and soils.

Keywords: dioxane, health risk, exposure, assessment, toxicology

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1. INTRODUCTION

1,4-Dioxane (synonyms paradioxane, 1,4-dioxacyclohexane) is a synthetic industrial chemical with a faintly pleasant odor that reportedly is found at many sites due to its widespread historical use. It is used in a variety of industrial organic products as a solvent, as a past stabilizing agent in chlorinated solvents (NHDES, 2011), and a byproduct of polyester manufacturing. 1,4-Dioxane also has been used in many products such as paint thinners, dyes, greases, and waxes. Residues may be present in manufactured food additives, food packaging materials, or on food crops treated with pesticides that contain 1,4-dioxane (USEPA, 2013a). It also is found in many household products. Several manufacturers recently announced that they met their 2013 Safety and Care Commitment to reduce traces of 1,4-dioxane in all baby and adult care products around the world (e.g., Johnson & Johnson, 2014).

Recent advances in analytical identification, along with increased awareness by regulators and reductions in some regulatory limits have caused 1,4-dioxane to emerge as a significant groundwater contaminant at many U.S. sites. 1,4-Dioxane has gained widespread attention from regulators as a result of its physical and chemical properties, including limited volatility, miscibility in water, extreme environmental mobility, and resistance to biological or abiotic degradation. Due to decreased usage and current pollution prevention practices, organizations such as the United States Air Force (USAF) have treated 1,4-dioxane as a legacy contaminant (Anderson et al., 2012).

Commercial production of 1,4-dioxane was first reported in the U.S. in 1951, though limited commercial quantities reportedly were available as early as 1929 (NTP, 2011). A patent application was filed for the use of 1,4-dioxane as a stabilizer in 1,1,1-trichloroethane (TCA) in 1954 (Doherty, 2000), and most historical reports link those two substances. Among the major chlorinated solvents, usage of trichloroethylene (TCE) was dominant in the 1960s and 1970s, followed by TCA usage in the 1970s up through the Montreal Protocol in 1996 when TCA use was limited as a degreaser (Mohr, 2013). Contrary to a variety of empirical reports, Mohr et al. (2010) concluded that 1,4-dioxane was not used as a stabilizer in TCE (only for TCA) for the following reasons:

(1) Smaller boiling point differences between 1,4-dioxane and TCE than between 1,4- dioxane and TCA;

(2) TCE is much more stable than TCA, and thus smaller quantities of stabilizer are required; and,

(3) Dow Chemical Co. reported that 1,4-dioxane was not used in its TCE formulations.

This conclusion was further supported when, in describing the history of TCE and TCA, Doherty (2000) did not list 1,4-dioxane as a stabilizer for TCE, but instead only mentioned its use as a stabilizer for TCA. Mohr et al. (2010) extended his point by analysis of chemical market reports showing that ~90% of all 1,4-dioxane produced in the U.S. was used as a stabilizer for TCA. On balance, however, the distinction is inconsequential for the purposes of this discussion, because it is well known that TCA and TCE were used in similar manufacturing situations at various times in history and both TCE and TCA, as well as several TCE degradation products (e.g., cis-1,2-dichloroethylene [cis-1,2-DCE]), are often found together at historical cleanup sites nationwide.

It is believed that 1,4-dioxane and TCE often are found together at these cleanup sites because TCE was released first and has had time to migrate a considerable distance and TCE is less prone to abiotic degradation than TCA or 1,4-dioxane, allowing 1,4-dioxane to "catch up" with the historical TCE plume (Zenker et al., 2003). In addition, it is well known that 1,4-dioxane will precede the TCA plume at some waste sites due to its miscibility in water (Anderson et al., 2012; Mohr, 2013).

1,4-Dioxane has been identified in less than 2% of U.S. hazardous waste sites (31 of 1,689; ATSDR, 2012), but it is likely to be present at others, yet not analyzed for or not reported due to elevated analytical detection limits associated with common laboratory methods (Adamson et al., 2014). The July 2014 Unregulated Contaminant Monitoring Rule (UCMR) report listed 1,715 samples greater than the minimum reporting limit (MRL) of 0.07 ug/L out of a little over 15,000 public water supply system (PWS) samples (USEPA, 2014b). Of these, 493 were greater than the reference concentration (as derived from the 2012 Health Advisory Tables) of 0.35 ug/L (10^{-6} risk) and none were greater than 35 ug/L (10^{-4} risk). Its relatively high boiling point (101.1 ^{o}C) can cause the concentration of 1,4-dioxane to increase in relative concentration compared to the principal solvents during the solvent vapor degreasing process which results in solvent losses, leading to possibly higher than anticipated groundwater concentrations of 1,4-dioxane (USEPA, 2006b). In October 2009, 1,4-dioxane was added to the final third Contaminant Candidate List (CCL) as one of 104 chemicals or chemical groups believed to have the potential to present health risks through drinking water exposure (USEPA, 2009).

As a result of its chemical structure, 1,4-dioxane is quite stable and relatively resistant to reactions with acids, oxides, and oxidizing agents such that concentrated acids and strong oxidizers under high temperature and pressure are required to break the ring structure (Mohr et al., 2010). A low potential for bioaccumulation and a low binding affinity for organic matter allows 1,4-dioxane to leach readily to groundwater from soils, a feature which causes groundwater to be the primary environmental medium of interest (Stickney et al., 2003; USEPA, 2006b; NTP, 2011). Many Potentially Responsible Parties (PRPs) are being required to modify existing remediation and/or monitoring plans to account for newly discovered, often very low, quantities of 1,4-dioxane in groundwater. The calculated health risks at a particular site are very specific and unique to the potential site exposure considerations. Thus, the discovery of 1,4-dioxane at a site may or may not affect the overall risk posed by aggregate site contamination. Because 1,4-dioxane plumes reportedly have been measured at lengths greater than the associated chlorinated solvent plume, with an area up to six times greater, the definition, capture, and remediation of a 1,4-dioxane plume can be considerably more challenging than is the case for other dissolved substances.

2. TOXICITY SUMMARY AND HEALTH GUIDELINES

2.1 Potential Noncancer Effects

Most of the toxicity information available for 1,4-dioxane is from laboratory animal studies, with only limited data from human exposures. The primary route of human exposure from environmental sources is ingestion, where 1,4-dioxane is well-absorbed. However, inhalation exposure may take place in the occupational setting as well. Two fatal occupational poisonings

were reported in the early to mid 1990's following (1) acute inhalation exposure at unknown concentrations and, (2) a high concentration (measured 208-650 parts per million [ppm]) acute inhalation and dermal exposure (USEPA, 2013b). These occupational exposures resulted in hemorrhagic nephritis and necrosis of the liver. Several human volunteer acute inhalation studies from the 1930's and 1940's showed irritation of the eyes, nose and throat, and slight vertigo following high level exposures (300-5500 ppm; USEPA, 2013b). Two later human volunteer studies, performed under ethical standards protocols, reported eye irritation following a lower acute exposure (50 ppm) and no increased symptoms at 20 ppm for 2 hours (Young et al., 1977; Ernstgard et al., 2006). No data were found regarding the health effects of human exposure via the oral route. Exposure via the dermal route results in much less absorption and therefore limits the extent of possible exposure (OEHHA, 1998; USEPA, 2010). There are no human studies regarding reproductive or developmental effects of 1,4-dioxane exposure. 1,4-Dioxane does not accumulate in the human body and is primarily metabolized to 1,4-dioxane-2-one and β-hydroxyethoxy acetic acid (HEAA), which are excreted in urine (USEPA, 2010; ATSDR, 2012).

In animals, liver and kidney degeneration and necrosis have been observed frequently in acute oral and inhalation studies as well as chronic oral exposure studies (USEPA, 2013b). Negative results were obtained from many mutagenicity and genotoxicity studies including the Ames assay using several *Salmonella* tester strains, *E. coli* assay, mouse lymphoma assay (MLA), chromosomal aberration, and sister chromatid exchange, while a few results have led some authors to conclude that 1,4-dioxane is weakly genotoxic at best (or worst; De Rosa et al., 1996; USEPA, 2013b). Dourson et al. (2014) recently has opined that if mutations are truly caused by 1,4-dioxane, it would be only at high, overtly cytotoxic doses, a view which was supported by Stott et al. (1981) years earlier when they determined that the lack of observed genotoxic activity by 1,4-dioxane, coupled with the presence of cytotoxicity at doses that result in tumor formation, suggests a nongenetic mechanism of liver tumor induction in animal studies.

The original and current oral reference dose (RfD) of 0.03 mg/kg•day was derived by The U.S. Environmental Protection Agency (USEPA) in 2010. Even though the primary study used to derive the current RfD was available, USEPA did not derive an RfD in the prior 1988 1,4-dioxane Integrated Risk Information System (IRIS) assessment. USEPA chose to use the Kociba et al. (1974) results as the principal study for the derivation of an RfD because the liver and kidney effects were considered the most sensitive with a no observable effect level (NOAEL) of 9.6 mg/kg•day in male rats (USEPA, 2013b). In addition, there were many noted strengths to that study compared to others, despite the one weakness of not reporting individual incidence data to allow for a benchmark dose (BMD) analysis. Instead, the NOAEL was divided by an uncertainty factor of 300 to account for animal-to-human extrapolation, for interindividual variability, and for perceived database deficiencies.

Dourson et al. (2014) concluded that while 1,4-dioxane causes liver tumors in rats and mice, it does so only at high doses through a mechanism of cytotoxicity followed by regenerative hyperplasia. Those authors recommended an RfD of 0.05 mg/kg•day to protect against this hyperplasia. In much the same fashion as that applied by USEPA in the case of chloroform during establishment of the trihalomethane standards in 2006, Dourson et al. (2014) used this RfD to calculate an MCL of 350 ug/L, based on a threshold mechanism of action. USEPA (2006a) concluded that the dose-response of chloroform is nonlinear and that carcinogenicity only results under high exposure conditions that lead to cytotoxicity and regenerative hyperplasia

and thus recommended a MCLG of 70 ug/L for chloroform (USEPA, 2006a). That evaluation was based on determining the Mode of Action (MOA), which was the main focus of the 2005 Guidelines for Carcinogen Risk Assessment (USEPA, 2005). At present, USEPA (2013b) has determined that the information is insufficient to unequivocally support the hypothesized nongenotoxic MOA for nasal and liver tumors (USEPA, 2013b). Presumably, credible publications like that of Dourson et al. (2014) will continue to keep the regulatory status of 1,4-dioxane in the forefront for USEPA and other state or federal agencies. To the extent that this nongenotoxic mechanism achieves greater acceptance, it will have a dramatic effect on the toxicity values and remediation guidelines associated with 1,4-dioxane, including the drinking water MCL value.

Early studies from Fairley et al. (1934) and Torkelson et al. (1974) were determined by USEPA to be inadequate for derivation of a reference air concentration (RfC) value for 1,4-dioxane. Fairley et al. (1934) studied exposure of rabbits, guinea pigs, rats, and mice to a variety of concentrations of airborne 1,4-dioxane. Only limited effects were observed in several species, though it isn't possible to calculate the air concentrations of exposure, due to the design of the experiment. Torkelson et al. (1974) found no significant difference between control rats and those exposed to approximately 111 ppm (~400 mg/m^3), 7 hours/day, 5 days/wk for two years. It wasn't until the Kasai et al. (2009) study that EPA developed the RfC of 0.03 mg/m^3 published in the 2013 Toxicological Assessment for 1,4-dioxane with an inhalation update (USEPA, 2013b). The RfC was derived by adjusting the selected point of departure (POD; 8.9 ppm, or approximately 32 mg/m^3) adjusted for continuous human exposure over a lifetime (which is highly unlikely) from results of a 2-year inhalation study that showed atrophy and respiratory metaplasia of the olfactory epithelium in male rats and adjusting it to reflect a human equivalent concentration (HEC; Kasai et al., 2009; USEPA, 2013b). An Uncertainty Factor of 1,000 was used to calculate the HEC, which equates to the RfC.

2.2 Potential Carcinogenic Effects

Two small human cohort studies conducted in the 1970's reported no conclusive evidence for a causal link between exposure to 1,4-dioxane and tumor formation (Buffler et al., 1978; USEPA, 2013b). The National Cancer Institute (NCI) performed studies in the late 1970's which showed that 1,4-dioxane induced hepatocellular adenomas in female Osborne Mendel rats, squamous cell carcinomas of nasal turbinates in both male and female Osborne Mendel rats, and hepatocellular carcinomas in both sexes of B6C3F1 mice (NCI, 1978). 1,4-Dioxane was first listed in the Second Annual Report on Carcinogens by the Department of Health and Human Services in 1981 as "reasonably anticipated to be a human carcinogen" and it continues to be listed as such (NTP, 2011). The International Agency for Research on Cancer (IARC; 1999) has classified 1,4-dioxane as a possible carcinogen to humans (Group 2B), based on inadequate evidence in humans and sufficient evidence in experimental animals, while ACGIH classifies it as an A3 confirmed animal carcinogen with unknown relevance to humans (ACGIH, 2014). In 1988, USEPA classified 1,4-dioxane as a B2 "probable carcinogen" with a numerical cancer slope factor (CSF) of 0.011 $(mg/kg•day)^{-1}$ derived from tumor incidence data for nasal squamous cell carcinoma in male rats exposed for 2 years in drinking water (USEPA, 2010). Under the most current Guidelines for Carcinogen Risk Assessment (USEPA, 2005), 1,4-dioxane is classified as "likely to be carcinogenic to humans" based on liver carcinogenicity in several animal species. A revised CSF of 0.1 $(mg/kg•day)^{-1}$ was derived based on the incidence of hepatocellular

adenomas and carcinomas in female mice exposed for 2 years to 1,4-dioxane in drinking water (Kano et al., 2009; USEPA, 2010). Based on results of newly available research, an inhalation unit risk (IUR) was derived in 2013, as listed in Table 1 (Kasai et al., 2009; USEPA, 2013b).

Table 1. Risk Values for 1,4-Dioxane

	CSF $(mg/kg•day)^{-1}$	IUR $(\mu g/m^3)^{-1}$	RfD (mg/kg•day)	RfC (mg/m^3)
1988	0.011	ND	ND	ND
2010	0.1	ND	0.03	ND
2013	0.1	5×10^{-6}	0.03	0.03

ND-Not determined

2.3 Drinking Water and Soil Guidelines

The USEPA has not established an enforceable Maximum Contaminant Level (MCL) for 1,4-dioxane in drinking water. For this reason there are numerous state guidelines, and one actual standard, that have been repeatedly amended. Despite the absence of a legally enforceable level in all states except Colorado, 1,4-dioxane can be subject to regulation through a variety of state-specific cleanup requirements (USEPA, 2006b). As an example of the changing guidance values, the California Department of Public Health (CDPH) in 1988 published an initial "notification level" of 3 µg/L based on the USEPA IRIS evaluation that same year, but subsequently revised its notification level to 1 µg/L in November 2010, following USEPA's revised recommendation (CDPH, 2011). Another example is Colorado, where the drinking water standard for groundwater started out in 2005 at 6.1 µg/L, followed by a decrease to 3.2 µg/L, with a further downward revision to 0.35 µg/L, which is the current value listed in regulation No. 41 (CDPHE, 2013). Table 2 shows the variety of regulatory concentrations across the states for 1,4-dioxane in drinking water, as well as some potentially applicable federal guidance values.

Beginning in 2010, when the Preliminary Remediation Goals (RPGs) or Regional Screening Levels (RSLs) were combined into one single table to be used nationally, the tap water screening value for 1,4-dioxane was set at 0.67 µg/L. In May 2014, this value was changed to 0.78 µg/L, principally due to changes in exposure assumptions that were made to the age-adjusted ingestion rate. The new assumptions that were used include a child ingestion rate of 0.78 L/day, adult ingestion rate of 2.5 L/day, adult body weight of 80 kg and adult exposure duration of 20 years. Both the dermal and the ingestion pathway are considered in this calculation, however the dermal pathway contribution is negligible to overall risk. EPA has calculated no volatilization factor (VF) for 1,4-dioxane, as according to their definition of a Henry's Law constant greater than or equal to 1×10^{-5} atm-m^3/mole and a molecular weight of less than 200 g/mole, 1,4-dioxane is not a volatile organic chemical (VOC). Therefore, there is no inhalation screening level contribution to the overall tapwater RSL of 0.78 µg/L, which is based upon potential carcinogenic effects. The Dourson et al. (2014) study suggests that the mechanism of action does not support such a restrictive guideline.

Table 2. Drinking Water Regulations & Guidelines for 1,4-Dioxane

State	*Regulatory Number*	*Value*	*Units*
California	Notification Level	1	µg/L
Colorado	Interim Standard	0.35	µg/L
Connecticut	Action Level	3	µg/L
Florida	Groundwater Cleanup Target Level (GCTL)	3.2	µg/L
Maine	Maximum Exposure Guidelines (MEG)	4	ppb
Massachusetts	Drinking Water Guidelines (ORSG)	0.3	µg/L
Michigan	Risk-based Screening Level (RBSL)	85	ppb
New Hampshire	Ambient Groundwater Quality Standard	3	µg/L
USEPA	Health Advisory Level (HAL)	0.35	µg/L
USEPA	Regional Screening Level (RSL)	0.78	µg/L
World Health Organization (WHO)	Drinking Water Guideline	50	µg/L
Wyoming	Water Clean up Level	0.85	µg/L

Sources: MECDC, 2012; MADEP, 2012; MIDEQ, 2012; CDPHE, 2013; FDEP 2005; CDPH, 2011; USEPA, 2011; USEPA, 2014a; WHO, 2005; WYDEQ, 2013.

The current USEPA residential soil screening level for 1,4-dioxane is 5.3 mg/kg, which changed slightly from the previous value, presumably due to the exposure parameter assumptions that changed with the 2014 RSL tables as shown in the tap water example above. This soil level incorporates exposure from all three potential environmental routes (ingestion, dermal, and inhalation), with the ingestion pathway contributing most to the overall carcinogenic RSL. The inhalation component is considered in that calculation due to theoretical particulate emissions, but in this instance the route is inconsequential. Table 3 compares some existing state soil guidance values with those of USEPA, including direct contact as well as the protection of drinking water from potential soil leaching, if applicable. The range is striking.

Table 3. Soil Regulations & Guidelines for 1,4-Dioxane

State	*Regulatory Name*	*Residential Direct Contact*	*Protection of Groundwater/DrinkingWater*	*Units*
California	Soil Screening Number	18	ND	mg/kg
Colorado	Groundwater Protection Values Soil Cleanup	23	0.0016	mg/kg
Florida	Soil Cleanup Target Level (SCTL)	23	0.01	mg/kg
Massachusetts	Method I Cleanup Standards	0.2	ND	mg/kg
Michigan	Risk-based Screening Levels	530	1.7	mg/kg
USEPA	Regional Screening Level (RSL)	5	1.6×10^{-4}	mg/kg
Wyoming	Cleanup Level	4.9	1.72×10^{-4}	mg/kg

Sources: OEHHA, 2010; CDPHE, 2014; FDEP, 2005; MADEP, 2012; MIDEQ, 2013; WYDEQ, 2013; USEPA, 2014a.

3. ANALYTICAL METHODS FOR DETECTION

One of the challenges associated with analytical detection of 1,4-dioxane is that the method has to be capable of separating low levels of an infinitely hydrophilic contaminant from water samples (Mohr et al., 2010). The complete water solubility (i.e., miscible) and thus poor purging efficiency of 1,4-dioxane requires modifications to existing methods in order to achieve the increased sensitivity necessary for the desirable lower detection limits. Purging at elevated temperatures allows more 1,4-dioxane to be removed from the water (USEPA, 2006b), and use of selected ion monitoring (SIM) can improve the sensitivity needed to detect the compound by mass spectrometry.

In the past, USEPA methods such as 8260B, 8270D, and 524.2 have been used for detecting 1,4-dioxane in water samples. In 2008, USEPA developed Method 522, which is a specific method for low detection limit analysis of 1,4-dioxane (USEPA, 2008). The Florida Department of Environmental Protection (FDEP) and some other state agencies have proven this method to be robust and to provide detection limits far superior to the other analytical methods evaluated, with the lone limitation being its narrow application to only one chemical (FDEP, 2010). Although

some laboratories may be able to make modifications to older methods in order to achieve sub ppb detection limits, such as isotope dilution (Draper, 2000) and large injection volumes, most work suggests that USEPA Method 522 is a better choice for detecting sub ppb levels of 1,4-dioxane in water. It is worth noting here that recent modifications and improvements to Method 8270D suggest that the method readily can achieve sub-ppb levels as well.

4. TREATMENT TECHNOLOGIES

The same properties that make 1,4-dioxane difficult to quantify at low concentrations in water samples also make it difficult to treat in groundwater remedial projects. Technologies used for chlorinated solvents often are not effective for treating 1,4-dioxane because of the differences in physical properties. Due to its high solubility and low tendency to adsorb to soils, 1,4-dioxane is well-suited for groundwater extraction and treatment (USEPA, 2006b; Moyer, 2008). Volatilization and sorption are not significant attenuation processes because of the low Henry's Law Constant (HLC), low octanol-water partition coefficient (K_{ow}), and infinite water solubility (Zenker et al., 2003). Due to it's low HLC it will likely remain dissolved in porewater making vadose soil vapor extraction (SVE) not very effective unless measures are taken to dry the soil (Mohr, 2013). The first highly successful *in situ* remediation using Electrical Resistance Heating (ERH) technologies have been shown to decrease 1,4-dioxane concentrations following 186 days of ERH operation, resulting in up to 99% removal (Oberle and Crownover, no date). The laboratory studies show that 1,4-dioxane is strippable at elevated temperatures; however, the authors conclude that there may be another mechanism causing the reductions in the field (Oberle and Crownover, no date). The energy requirements and cost of such an approach can be daunting as well.

Due to its heterocyclic nature and two ether linkages, 1,4-dioxane is resistant to abiotic and biologically mediated degradation, although some laboratory results have shown that biodegradation may occur under specified conditions (Zenker et al., 2003). Chiang et al. (2008) showed decreasing concentrations of 1,4-dioxane in groundwater during Monitored Natural Attenuation (MNA) at a site where the principal source of contamination had been removed; however, MNA may have limited success at removing large quantities of 1,4-dioxane. Steffan (2007) found that several microorganisms were able to degrade 1,4-dioxane via cometabolism during growth on propane or tetrahydrofuran (THF), but it was not consistently degraded in microcosms created with samples from various 1,4-dioxane contaminated aquifers. As such, they concluded that biological treatment and MNA are unlikely to be successful as remedial alternatives for 1,4-dioxane in groundwater (Steffan, 2007). In addition, more recent research has shown that consideration of the presence of other chlorinated solvents, as well as transition metals, is warranted due to their possible ability to further inhibit biodegradation further (Mahendra et al., 2012; Pornwongthong et al., 2014).

Advanced oxidation processes (AOPs) appear to be most commonly used for full scale *ex situ* 1,4-dioxane treatment (Mohr et al., 2010). AOPs are available for aboveground treatment of 1,4-dioxane from groundwater, and can be used alone or in combination with other remediation processes (USEPA, 2006b). Two common AOPs used for 1,4-dioxane treatment are hydrogen peroxide (H_2O_2) with ultraviolet (UV) light, and H_2O_2 with ozone (DiGuiseppi and Whitesides, 2007). Limitations of using AOPs include the formation of bromate, if the contaminated water contains bromide, as well as other decomposition products. There is a limited ability to handle

concentrations of 1,4-dioxane above 1 milligram per liter (1 mg/L) unless multiple units in a series are utilized (USEPA, 2006b).

Some firms offer treatment technologies which incorporate soil vapor extraction, air sparging, air stripping, flushing, and enhanced bioremediation/oxidation into a single groundwater well with a minimum diameter of 4 inches. Odah et al. (2005) showed a 70% reduction of 1,4-dioxane at a distance of 10 feet from a treatment well and 91% at a distance of 20 feet from the treatment well within three months.

Phytoremediation technologies are also being explored; however, it is unclear whether plants are able to take up 1,4-dioxane in appreciable amounts (USEPA, 1999; USEPA, 2013a). Aitchison et al. (2000) found that hybrid poplar trees, which were chosen because they are easily propagated, develop deep root systems, exhibit high water uptake rates, and are tolerant to high levels of organics, were effective at removing 1,4-dioxane from water samples and soil samples in the laboratory. Most of the 1,4-dioxane taken up by the roots was then volatilized by transpiration from the leaf surfaces (Aitchison et al., 2000).

5. CONCLUSION

PRPs increasingly should expect laboratories to be able to detect 1,4-dioxane concentrations in the sub ppb range in order to meet the low guidelines in this range. Although its mere presence may not translate into a significant human health risk, its occurrence is getting attention from a regulatory perspective. The discovery of 1,4-dioxane at existing sites, or where cleanup is already being performed for other chemicals of concern, can cause extensive problems and delays for PRP's. These can include increased monitoring, as well as costs associated with more sensitive analytical techniques required to detect 1,4-dioxane in the sub ppb range. It is important for PRPs to be aware that if VOCs, especially TCE or TCA, are found at a site they should consider analyzing the samples for 1,4-dioxane to ensure that the chosen treatment strategy will effectively remove all contaminants of concern from the site and will likely not require further treatment years later. Likewise, the mere presence of a contaminant at low levels does not mean that it will represent a significant human health risk.

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ENVIRONMENTAL OCCURRENCE AND REGULATION OF HISTORICAL FUMIGANTS 1,2-DIBROMOETHANE AND 1,2-DIBROMO-3-CHLOROPROPANE

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ABSTRACT

1,2-Dibromoethane (EDB) and 1,2-dibromo-3-dichloropropane (DBCP) were used historically from the 1950s to the early 1980s on a widespread basis as soil fumigants for control of nematodes on a variety of fruit and vegetable crops. EDB also was used as a lead scavenging agent in leaded gasoline. Use of these chemicals for agricultural applications was discontinued by the U.S. Environmental Protection Agency (USEPA) based on concerns related to potential adverse human health effects. These chemicals are infrequently detected in soil as a result of their volatility and limited solubility in water. However, they are occasionally detected at low concentrations in groundwater. Both chemicals are regulated as drinking water contaminants with allowable limits set at very low concentrations in the parts per trillion (ppt) range. Half-lives in groundwater vary widely depending on local groundwater conditions. Even in areas where long term application of these chemicals was practiced, only low concentrations in groundwater may currently be detected. However, the extremely restrictive environmental guidelines can cause these substances unexpectedly to rise to potential significance at some sites, even several decades after the cessation of application. Product use, toxicology, regulatory guidance, and risk-based considerations are discussed.

Keywords: EDB, 1,2-dibromoethane, ethylene dibromide, DBCP, 1,2-dibromo-3-chloropropane, dibromochloropropane,pesticide, soil, groundwater

1. INTRODUCTION

The large majority of 1,2-dibromoethane (ethylene dibromide; EDB; see Figure 1 for chemical structure) is synthetically produced, but it may occur naturally in the ocean in very small quantities (ATSDR, 1992a). The major use of EDB was as a gasoline additive to scavenge lead oxides formed during the combustion of leaded gasoline in automobiles beginning in the 1920s. Use of EDB for this purpose has declined since the U.S. Environmental Protection Agency (USEPA) phased out the use of tetraethyl and tetramethyl lead beginning in the 1980s and banned the use of leaded gasoline in automobiles in 1996 (Redding, 2011; ATSDR, 1992a; ATSDR, 2007; Federal Register, 1996). EDB also was used as a soil fumigant from 1940 to 1983 when its use for this purpose was banned by USEPA (ATSDR, 1992a; Federal Register, 1983). Other minor applications for the chemical include treatment of felled logs for bark

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beetles, termite control, control of wax moths in beehives, spot treatment of milling machinery, Japanese beetle control in ornamental plants, as a chemical intermediate for dyes and resins, and as a general solvent for waxes, gums, and dyes (ATSDR, 1992a; HSDB, 2005a). A chemically related substance, 1,2-dibromo-3-chloropropane (dibromochloropropane; DBCP; see Figure 1 for chemical structure) is a synthetic chemical not found naturally in the environment (ATSDR, 1992b). The principal use of DBCP was as a soil fumigant and nematicide from 1955 to 1977 when its use for this purpose was banned by USEPA (ATSDR, 1992b; Federal Register, 1977; Federal Register, 1979; IARC, 1979). DBCP also is used as an intermediate in the synthesis of organic chemicals, such as the brominated flame retardant tris(2,3-dibromopropyl)phosphate (ATSDR, 1992b; HSDB, 2005b).

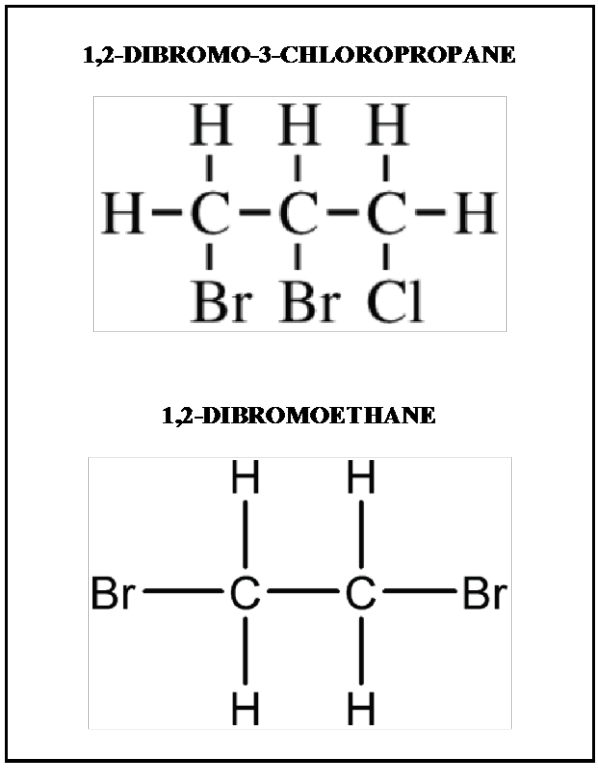

Figure 1. Chemical structures of DBCP and EDB

Because both substances are no longer produced, most human exposures to EDB and DBCP are expected to occur via contaminated environmental media, including drinking water (ATSDR, 1992a, 1992b), and the more recent scientific literature predominantly addresses that route of exposure. In areas where DBCP was used as a soil fumigant, it may still be present in soil and groundwater at low concentrations (ATSDR, 1992b). The presence of EDB and DBCP in groundwater used as drinking water sources is regulated at extremely low concentrations. As a result, their presence in areas where they were formerly used as pesticides has the potential to cause concern regarding the potential for health effects from exposure to them. Claims of potential health impacts have led to legal action regarding past misuse of these chemicals.

2. REPORTED ENVIRONMENTAL SOIL LEVELS

Information on soil concentrations of EDB and DBCP is limited. Because both chemicals are volatile, they are expected to dissipate from shallow soil relatively quickly following application as agricultural pesticides, leaving only low level residues.

Reported soil concentrations of EDB range from <0.00005 to 0.0311 mg/kg (Zalkin et al., 1984; Duncan and Oshima, 1985; McConnell et al., 1984; Oki and Giambelluca, 1989). The maximum value in this concentration range is lower than the USEPA residential soil Regional Screening Level (RSL) of 0.036 mg/kg, based on the potential carcinogenic effects of EDB (USEPA, 2014). Reported soil concentrations of DBCP range from <0.000025 to 0.005 mg/kg (Carter et al., 1984; Oki and Giambelluca, 1989; Peoples et al., 1980). The maximum value in this concentration range is lower than the USEPA residential soil RSL of 0.0053 mg/kg, based on the potential carcinogenic effects of DBCP (USEPA, 2014).

Based on available soil sample data collected during the 1980s, soil concentrations of EDB or DBCP are not expected to occur at levels of health interest, even at locations where these chemicals were applied routinely for pest management prior to the late 1970s and early 1980s when their use for this purpose was banned.

Biological degradation of EDB, as with abiotic processes described previously, is slow relative to volatilization. Bacterial degradation has been reported for aerobic soils, but is limited under anaerobic conditions. EDB reportedly can be detected in soils for a decade or more after application as a fumigant (ATSDR, 1992a). For DBCP, soil biodegradation may occur under some conditions, though the prediction of those conditions is difficult and variable (ATSDR, 1992b), and the process is very slow to progress.

3. REPORTED ENVIRONMENTAL GROUNDWATER LEVELS

3.1 1,2-Dibromoethane

From a point of regulatory interest, EDB still occasionally is detected at concentrations exceeding its drinking water MCL (0.05 ug/L; 50 parts per trillion or ppt; USEPA, 2009). EDB concentrations have been monitored in a number of states including California, Washington, Georgia, Oregon, Hawaii, Indiana, South Carolina, and Connecticut. Between 2004 and 2011, CalEPA (2013) detected EDB in groundwater at concentrations ranging from 0.01 to 0.17 ug/L.

Landmeyer and Campbell (2010) reported concentrations of EDB in public wells in 2006-2008 ranging from <0.03 to 9.1 ug/L and in private wells ranging from <0.01 to 2.5 ug/L. For groundwater samples collected in 2007, Redding (2011) detected EDB at concentrations ranging from <0.019 to 0.27 ug/L. Kaluarachchi et al. (2002) reported a generally decreasing trend in EDB concentrations for groundwater in Washington between 1984 and 1999 with a maximum of 6.1 ug/L at one location in 1986 which decreased to 0.68 ug/L in 1998. In Hawaii, EDB concentrations in groundwater also have been generally declining from a reported concentration of 0.07 ug/L in 1985 to <0.04 ug/L in 2005 (HSDOH, 2006).

EDB reportedly has a moderately low solubility in water (~4,000 mg/L; HSDB, 2005a), though that value clearly is far greater than its acceptable drinking water concentration. Coupled with its volatility, these two properties favor losses from water supplies when exposed to air.

Reported hydrolysis half lives of EDB in groundwater cover a wide range, but it generally is considered to be a persistent environmental chemical. Pignatello and Cohen (1990) reported hydrolysis half lives for EDB of 1.5-2 and 8 years at 20^{o} C, and 2.2 and 15 years at 15^{o} C. Vogel and Reinhard (1986) reported the hydrolysis half life of EDB as 2.5 years at pH 7 and 25^{o} C. A half life of 13.2 years at pH 7 and 20^{o} C also has been reported (HSDB, 2005a). Given that the last permitted use of EDB as a soil fumigant occurred over 30 years ago, it is likely that concentrations of EDB in groundwater have been significantly reduced through this process even assuming the longest reported hydrolysis half life value (15 years).

3.2 1,3-Dibromo-3-chloropropane

From a regulatory perspective, DBCP is still occasionally detected at concentrations above its MCL (0.2 ug/L, 200 ppt; USEPA, 2009). DBCP concentrations have been monitored in a number of states including California, Washington, Hawaii, and South Carolina. Between 2004 and 2011, CalEPA (2013) detected DBCP in groundwater at concentrations ranging from 0.01 to 2.03 ug/L. Landmeyer and Campbell (2010) reported concentrations of DBCP in public wells ranging from <0.03 to 0.69 ug/L and in private wells ranging from <0.03 to 5.6 ug/L. For groundwater samples collected in 2007, Redding (2011) detected DBCP at concentrations ranging from <0.019 to 0.071 ug/L. DBCP concentrations in groundwater monitored in Hawaii in 2005 ranged from <0.04 to 0.36 ug/L.

DBCP has a relatively low solubility in water (1,230 mg/L; ATSDR, 1992b). DBCP is fairly mobile in soil, particularly in sandy soil with low organic carbon content (ATSDR, 1992b). DBCP applied as a pesticide that did not volatilize to the atmosphere is likely to have been transported to groundwater by rainwater leaching processes (ATSDR, 1992b).

Hydrolysis half lives for DBCP in groundwater are variable and have been reported at 6.1 years (at pH 7.8 and 21.1^{o} C; Deeley et al., 1991), and 38 years (at pH 7 and 25^{o} C) and 141 years (at pH 7 and 15^{o} C; Burlinson et al., 1982). Other reported half lives for DBCP range as low as 2 to 6 years (Burow et al., 2007). Based on the wide range of reported half lives for DBCP in groundwater, it is difficult to determine what impact hydrolysis would have with regard to the current groundwater concentrations of DBCP following its discontinued use in the late 1970s.

4. HEALTH EFFECTS OF EDB AND DBCP

Health effects from exposure to EDB and DBCP have been reported in animals and humans. Some animal studies on the health effects of exposure to EDB and DBCP have reported significant developmental and other adverse effects at high exposure levels. However, health studies involving human exposures have demonstrated varying and mostly inconclusive correlations.

There is a limited amount of human health information available regarding the effects of EDB. Inhalation of EDB in occupational settings has been reported to cause significant decreases in sperm count and the number of viable and motile sperm, while increasing sperm morphological abnormalities (WHO, 1999). Ingestion of high concentrations of EDB can cause liver necrosis and kidney lesions as well as ulceration of the mouth and throat (ATSDR, 1992a). EDB is a mild central nervous system depressant and drowsiness has been reported following ingestion and inhalation (ATSDR, 2014). There is evidence to suggest that EDB is rapidly broken down and readily removed from the body, indicating that it is not likely to accumulate in the body to any significant extent (ATSDR, 1992a; Kaluarachchi et al., 2002).

USEPA (2012b) has published an inhalation Reference Concentration (RfC) of 9 ug/m^3 for EDB and an oral Reference Dose (RfD) of 0.009 mg/kg•day. The International Agency for Research on Cancer (IARC) has classified EDB as probably carcinogenic to humans (Group 2A) based on inadequate evidence in humans and sufficient evidence in experimental animals (WHO, 1999). USEPA (2012b) has classified EDB as "likely to be carcinogenic to humans", based on inadequate data in humans and sufficient data in animals, and developed an oral slope factor of 2 $(mg/kg•day)^{-1}$ and a drinking water unit risk of 6E-05 $(ug/L)^{-1}$.

Although exposure to DBCP has been studied mostly in occupational settings, there is some human health information regarding the effects of exposure to DBCP in drinking water. Workers in chemical factories producing DBCP who breathed high levels (2,800 to 9,670 ug/m^3) of DBCP showed damage to male reproductive ability (ATSDR, 1992b; CalEPA, 1999). No reproductive effects were reported in male workers exposed to 9.67 ug/m^3 of DBCP in air. DBCP also has been reported to cause headache, nausea, lightheadedness, and weakness at air concentrations of about 3,870 ug/m^3 (ATSDR, 1992b). Exposure to DBCP in drinking water that contained concentrations ranging from 0.004 to 5.75 ug/L did not result in any increases in the number of birth defects or changes in newborn sex ratios (ATSDR, 1992b; CalEPA, 1999). An epidemiological study in a California population exposed to 0.004 to 5.75 ug/L DBCP in drinking water found no increase in leukemia incidence. Another study found no correlation between exposure to DBCP in drinking water and mortality rates for gastric cancer or leukemia (ATSDR, 1992b).

Although epidemiological data have not demonstrated that DBCP is carcinogenic in humans, animal evidence clearly shows that DBCP is carcinogenic in mammals (CalEPA, 1999). Based on inadequate evidence in humans and sufficient evidence in experimental animals, IARC has classified DBCP as possibly carcinogenic to humans (Group 2B; WHO, 1999). The twelfth edition of the Report on Carcinogens of the National Toxicology Program (NTP, 2011) has determined that DBCP can be reasonably anticipated to be a human carcinogen based on sufficient evidence in animal studies. USEPA (2012a) is reviewing the health information for DBCP in order to develop a cancer risk evaluation for this chemical. USEPA (2012a) has, however, published an RfC of 0.2 ug/m^3 for DBCP and ATSDR (1992b) has derived an intermediate duration oral Minimal Risk Level (MRL) for DBCP of 0.002 mg/kg•day.

A summary of agency exposure guidance concentrations and standards is presented in Table 1.

Table 1. Media Exposure Guidelines for EDB and DBCP.

Agency	Guideline	EDB	DBCP
USEPA	Oral RfD Inhalation	0.009 mg/kg•day	NA
	RfC Cancer Slope	9 ug/m^3	0.2 ug/m^3
	Factor	2 (mg/kg•day)$^{-1}$	NA
	DW Unit Risk	6E-05 (ug/L)$^{-1}$	NA
	Tap Water RSL MCL	0.0075 ug/L 0.05 ug/L	0.00033 ug/L 0.2 ug/L
ATSDR	Interm. Oral MRL	NA	0.002 mg/kg•day

RfD - Reference Dose
RfC - Reference Concentration
DW - Drinking Water
RSL - Regional Screening Level
MCL - Maximum Contaminant Level
MRL - Minimal Risk Level
NA - Not Available

5. RISK-BASED VS LEGAL CONSIDERATIONS

Although EDB and DBCP may be present in groundwater at concentrations that exceed regulatory guidelines (e.g., MCLs, RSLs), the potential for adverse health effects depends on the extent to which sufficient exposure may be possible. Historically, groundwater concentrations of both these chemicals have exceeded regulatory guidelines in a significant number of wells that have been sampled. However, detected concentrations have been reported to vary substantially from one well to another and for a given well over time. There also are reports that detected concentrations have decreased over time, based on long term monitoring of groundwater. Detected concentrations many times fluctuate inconsistently from one sampling event to the next so that it is not clear whether potential exposures are of realistic health interest.

The extent to which groundwater remediation is appropriate or legal actions related to cleanup are justified depends directly on an evaluation of the potential for adverse health effects based on risk assessment procedures. Discoveries of groundwater concentrations exceeding regulatory levels have led to legal action regarding cleanup, as well as allegations related to perceived health effects. Such claims are rarely substantiated, though it is difficult to separate statements made in the literature related to occupational exposures vs environmental exposures.

6. CONCLUSION

In many cases, the very low regulatory guidance concentrations drive site cleanup decisions even though EDB/DBCP concentrations may not be of human health interest from a realistic exposure perspective. Consideration of cleanup concentrations based on reasonable site-specific exposure scenarios and health risks is warranted.

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REMEDIATION OF BISPHENOL A WATER CONTAMINANTS BY REUSING ACTIVATED CHARCOAL FILTERS

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ABSTRACT

Endocrine disruptors (EDCs) are chemicals that interfere with the endocrine system. Bisphenol A (BPA), a common EDC found in plastic bottles and soda cans, is a synthetic estrogen that feminizes organisms. Efforts have been made to eliminate BPA from the water supply by using new filtration methods, but most are too expensive or ineffective. Experiments were conducted using activated charcoal filters and varying concentrations of BPA in two methods: consecutive rounds of batch use and continuous use. Our hypothesis was that activated charcoal would be a reliable method of filtration that would withstand repeated challenges of BPA. The concentrations of the BPA solutions were measured by UV absorption using a spectrophotometer after establishing a standard curve to determine the linear range of the assay. Sand filters, which are commonly used in water treatment facilities, were tested as a control. We found that both consecutive rounds of filtration and continuous use consistently removed 85% to 99% of the BPA. No statistical difference in removal was observed between consecutive use and continuous use when paired t-tests were performed ($p >> 0.05$, $\alpha = 0.05$). The sand filters were largely ineffective. These results demonstrate that activated charcoal is an effective, economical method to filter BPA from water, especially wastewater, where BPA is commonly found and left unfiltered.

Keywords: endocrine disruptors, bisphenol A, activated charcoal, filtration, water treatment

1. INTRODUCTION

Endocrine disrupting compounds (EDCs) are chemicals that act as either hormone receptor agonists or antagonists. They are commonly used in plastics, cosmetics, and pesticides and other common household items. The synthetic estrogen bisphenol A (BPA) is used in polycarbonate plastic containers, such as reusable water bottles, in thermal paper used for receipts, and in the resin lining soda cans. BPA is widely produced worldwide, with more than 6 billion pounds produced annually and as much as 100 tons of it released into the atmosphere (Vandenberg L et al., 2009). BPA also leaches readily from plastic so it is common for BPA to be present in food and water, posing a risk to humans.

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BPA acts as an estrogen receptor agonist, and effects are especially observed when exposure occurs prior to birth or early in life, with signs of exposure appearing during puberty (Vandenburg L et al., 2009). In one study, BPA was found to offset the reproductive axis of rats when exposed to BPA pre- and early post-natally (Gámez JM et al., 2014); in another it was reported that BPA affected neural circuitry related to male sexual behavior in the rats (Picot M et al., 2013). These effects may also be multi-generational and epigenetic on a human-relevant dose level (Boudalia S, et al. 2014). In humans, BPA exposure is associated with several physiological issues, including obesity, endometrial hyperplasia, miscarriages, sterility, and polycystic ovarian syndrome (Vandenberg L et al., 2009).

In addition to the potential to harm humans, BPA may harm wildlife and may be responsible for the observed feminization of male aquatic wildlife (Kidd K et al., 2007). EDCs are believed to "interfere with the functioning of receptors whose normal role is to mediate the effects…of hormones" (Colburn T et al., 1993). They cause various abnormalities in wildlife exposed to them, such as thyroid abnormalities in birds and fish, decreased fertility, demasculinization and/or feminization of male wildlife, and altering immune functions (Colburn T et al., 1993).

Some wastewater treatment plants currently use sand filters in addition to treatment by microorganisms, both of which are ineffectual at removing EDCs, especially BPA, because of the hydrophilic nature of EDCs. Sand filters, for example, typically have a <1% removal effectiveness rate. This inadequate filtration leaves a median concentration of 127 ng/L (557 pM) (Nakada N et al., 2007). However, these low concentrations are deceptively reassuring because EDCs are extremely potent even in small doses. BPA produces adverse effects on cells and organisms beginning at 1 pM. Given the median effluent concentration of BPA of 557 pM, the BPA present in sewage effluent currently poses a risk to the environment (Welshons WV et al., 2006). The danger of the presence of EDCs in our water, especially BPA, can be avoided by improvements in water filtration methods. Some treatment plants have begun implementing methods such as ozone treatment in addition to sand filtration. While this is an improvement, ozone treatment is often expensive to implement and maintain. Another promising method of filtration of EDCs is with activated charcoal, which consists of porous carbon particles.

The purpose of this study was to determine the effectiveness of an activated charcoal filter with 1) multiple rounds of filtration and 2) continuous filtration of the BPA solution. The findings of this study could support a range of potential applications in use of activated charcoal as an economical and effective method of filtering BPA on a large scale, like wastewater treatment, and on a smaller scale, such as in portable water bottles.

2. MATERIALS AND METHODS

2.1 Determining the Absorption Spectrum of BPA

To prepare solutions of BPA, 0.01 moles of BPA (Sigma-Aldrich) were dissolved in 10 mL of ethanol. 10-fold serial dilutions were then performed by taking 1 mL of the sample and adding 9 mL of deionized water. Dilutions were performed until a range of 1 M to 1.0×10^{-7} M was achieved. The successive two solutions from the original 1 M, from 1 M to 1.0×10^{5} M, and from 1.0×10^{5} M to 1.0×10^{4} M were diluted using ethanol to prevent precipitation of the BPA in water. The remaining dilutions were performed with deionized water. Samples were then separately

placed in a spectrophotometer (Thermo Scientific Nanodrop 2000c) in a quartz cuvette and absorbances for each concentration were obtained. The absorbance at 279 nm, which was the wavelength of peak absorbency, was then used to construct a standard curve and to make calculations.

2.2 Establishing a Standard Curve for BPA

Certain concentrations of solutions were eliminated from testing after yielding absorbances that were either below or above the range of the assay. The remaining solutions, between concentrations of 1000 µM to 5.0 µM, were then serially diluted two-fold twice by combining 5 mL of sample solution and 5 mL of deionized water. This yielded a total of six dilutions, each of which was tested in the spectrophotometer. A standard curve was constructed in Microsoft Excel by graphing the concentrations of the solutions and the absorbances.

2.3 Chromatography Column Sand Filter

0.1 g of sand was placed in each of six chromatography columns. 1 mL of each concentration tested was then loaded onto each column, filtered through the sand and samples collected in test tubes. Each of the filtered solutions was then measured in the spectrophotometer for its absorbance and the concentrations calculated using the standard curve.

2.4 The Activated Charcoal Batch Assay

3 g of finely ground activated charcoal (Sigma-Aldrich) were added to 3 mL of each concentration being tested in separate test tubes for each sample (Wang J and Field J, 2011). The samples were shaken for 30 minutes at 25°C (New Brunswick Series 25 Incubator Shaker) and centrifuged for 10 minutes (Beckman J6-HC centrifuge). The supernatant was collected from each sample and centrifuged for 10 minutes at 1500 rpm to remove any remaining activated charcoal. Each sample solution was then measured in the spectrophotometer for its absorbance and the concentrations calculated based on the standard curve of BPA absorbance.

2.5 Repeated Activated Charcoal Batch Assay

The activated charcoal from the first round of filtration was recovered from the test tubes and allowed to dry at 25°C in the shaking apparatus. Each sample of activated charcoal from the respective solutions was then weighed to determine how much had been recovered. The recovered activated charcoal was applied to solutions of the respective concentrations in ratios of 1 mL of solution for every 0.1 g of activated charcoal recovered. This was done to ensure a consistent ratio of activated charcoal to solution between the different concentrations, as each concentration had varying amounts of activated charcoal recovered from the test tubes depending on the success of the recovery. The samples then underwent the same procedure as was followed in the first round of batch filtration. The recovery of the filter and procedure of the third round of filtration was carried out similarly.

2.6 Column Chromatography Activated Charcoal

0.1 g of finely ground activated charcoal was placed in each of six chromatography columns. Six 1 mL solutions of BPA ranging in concentration from 1000 µM to 5.0 µM was then placed each column. 1.5 µL tubes were placed beneath each column to collect the filtered BPA solutions. The collection tubes were removed and replaced with new ones, and 1 mL of the respective solutions was applied to each column at hourly intervals. The filtered BPA solutions were each measured for their absorbances. The concentrations of each resulting solution were determined using the standard curve of absorbance constructed earlier. The process was carried out for a total of 4 hours, with a total of 4 mL of solution having been filtered through each column.

3. RESULTS

3.1 An Assay for BPA

We used absorbance spectroscopy to develop an assay for BPA using dilutions in the range of 1000 µM to 5.0 µM (Figure 1).

There are notable peaks at 230 nm and 279 nm, but for the range of concentrations tested (1000 µM to 5.0 µM), the latter was more consistent and offered a wider range of testing. For all subsequent experimentation, the spectrophotometer measured absorbance at 279 nm.

3.2 Standard Curve of BPA in Water

A standard curve for BPA in water was constructed from absorbances of the dilutions. Additionally, because of the minimum detectable range of 0.02, all absorbencies obtained below that value were assumed to be ≤0.02. The percentage error of the spectrophotometer is ± 2%.

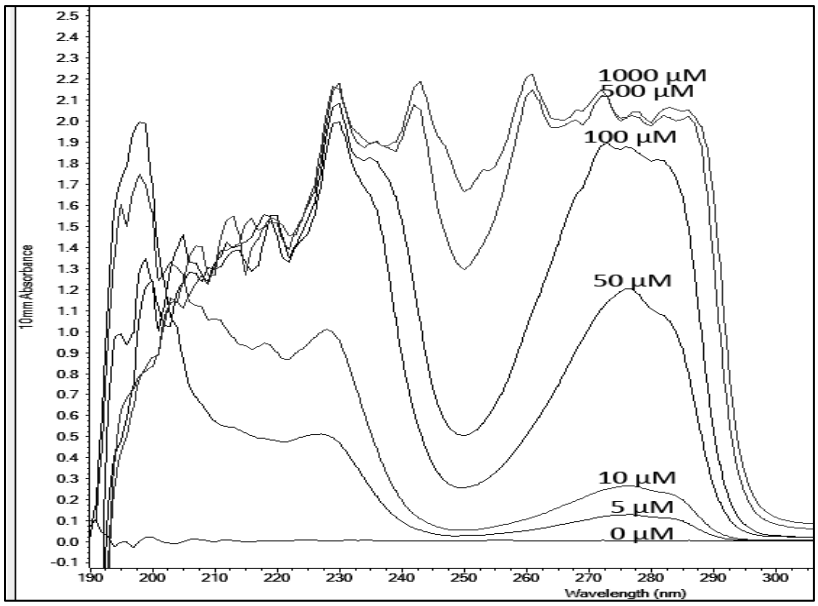

Figure 1. BPA, ranging in concentration from 5 µM and 1000 µM as indicated, was dissolved in ethanol and diluted with water below 0.1 M and tested in a spectrophotometer. Peaks at 230 nm and 279 nm are indicated.

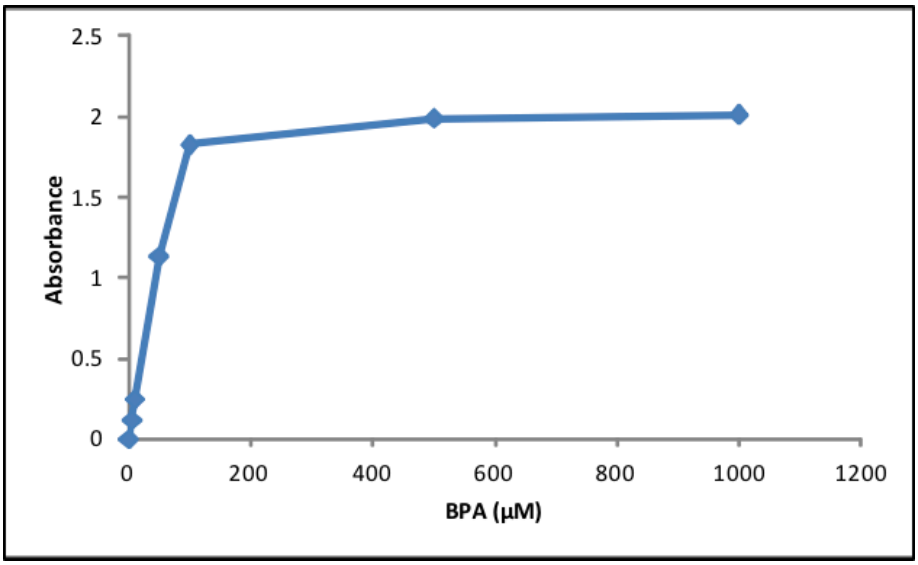

Figure 2. Absorbance of BPA standard curve for range of prepared solutions between 5 µM and 1000 µM. The equation used for calculations was taken from the linear relationship of absorbance and concentration in the range 5 µM and 100 µM, as indicated in Figure 3.

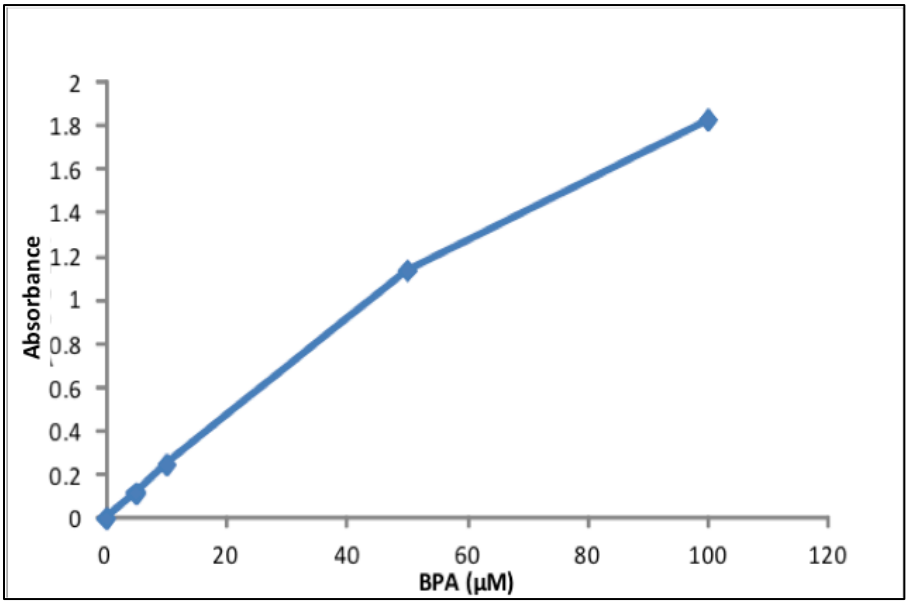

Figure 3. Absorbance of BPA standard curve for range of concentrations between 5 µM and 100 µM. Absorbances for concentrations greater than 100 µM were excluded because of the saturation the spectrophotometer above that concentration.

Figure 3 displays the relationship between the concentration of BPA and the absorbance at 279. This relationship was used for all further calculations of filtered concentrations based on the absorbances obtained. Because the minimum detectable absorbance was 0.02, any absorbance obtained below that value was assumed be ≤0.02, which is equivalent to a concentration of $\leq 6.04\times10^{-1}$ µM, based on the established standard curve.

3.3 Sand Filtration

As sand filters are commonly used in water treatment plants, we tested if BPA could be removed using a chromatography column filled with sand. The sand filtration was largely ineffective, except at the highest concentration of 100 µM, but only with a percent removal rate of 7.80% (Figure 4). The remaining concentrations had a negligible effect at removing BPA.

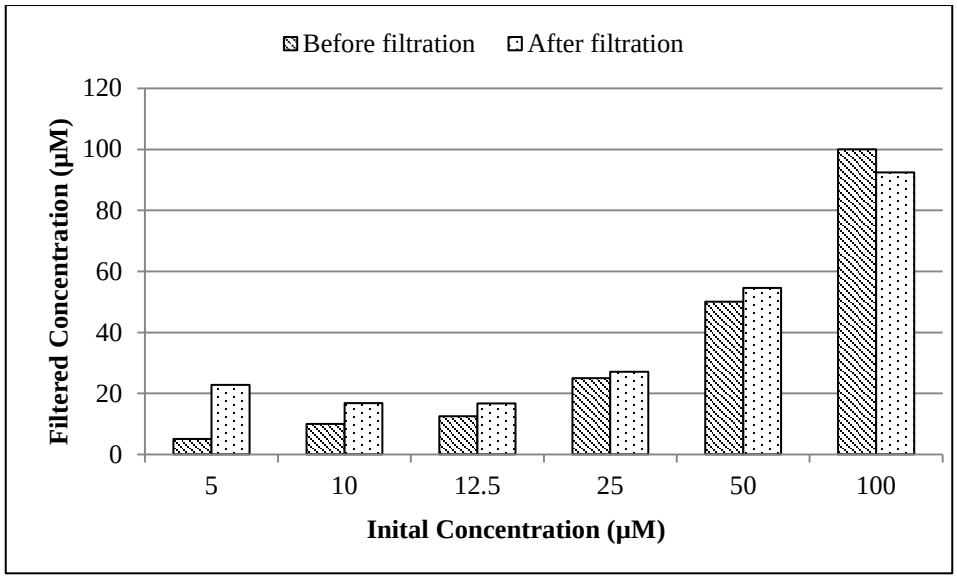

Figure 4. Filtration of BPA was largely unsuccessful using sand column chromatography.

3.4 Activated Charcoal Filtration and Repeated Use of Charcoal

Because sand filters were ineffective, we tested activated charcoal. Since charcoal filters may be reused and left to dry in real world situations, we also tested if the activated charcoal could withstand repeated drying cycles. To carry out the cycling, used activated charcoal from each round of filtration was dried at room temperature to exclude the possibility of full restoration of the charcoal, a process that involves heating the used charcoal to remove filtered substances. The charcoal recovered in each round of filtration varied, but the ratio of 0.1 g charcoal for every 1 mL of solution was maintained each time. As an example, Table 1 illustrated the values for charcoal recovered and amount of solution applied for the third rounds of filtration.

Table 1. Activated Charcoal Recovered from Second Use of Batch Filter

Concentration (µM)	Charcoal Recovered (g)	% Charcoal Recovered (of 0.3 g)
5	0.15	50%
10	0.26	87%
12.5	0.31	103%
25	0.33	110%
50	0.25	83%
100	0.05	17%

3.5 Effectiveness of Activated Charcoal Batch Filtration

We found that in the initial and all subsequent uses of a charcoal filter, the percent removal of the BPA was in the range of 88% to >99% (with ±2% error due to the spectrophotometer measurement capabilities). In the concentrations between 5 µM and 100 µM, the BPA removal rate was consistently ≥99% in all three rounds of filtration. As an example, Table 2 illustrates the percentage removal of BPA in the round of batch filtration at each concentration. Results obtained for all three rounds of filtration can be found in Figure 5 and Figure 6.

Table 2. Comparison of Concentration and Absorbance Before and After Activated Charcoal Batch Filtration (3rd time)

Initial Concentration (µM)	Initial Absorbance (abs)	Final Absorbance (abs)	Minimum Accurate Absorbance (abs)	Final Concentration (µM)	Removal of BPA (%) ±2%
5	0.117	0.008	<0.020	<0.604	>88%
10	0.247	0.003	<0.020	<0.604	>94%
12.5	0.367	0.008	<0.020	<0.604	>95%
25	0.612	0.003	<0.020	<0.604	>98%
50	1.135	0.010	<0.020	<0.604	>99%
100	1.824	0.013	<0.020	<0.604	>99%

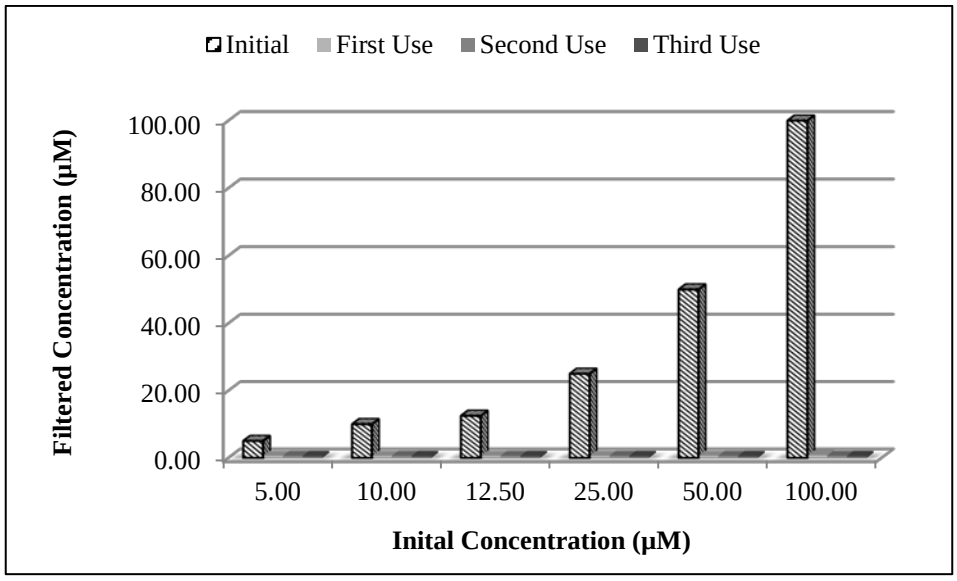

Figure 5. Concentrations of BPA with Three Rounds of Activated Charcoal Batch Filtration.

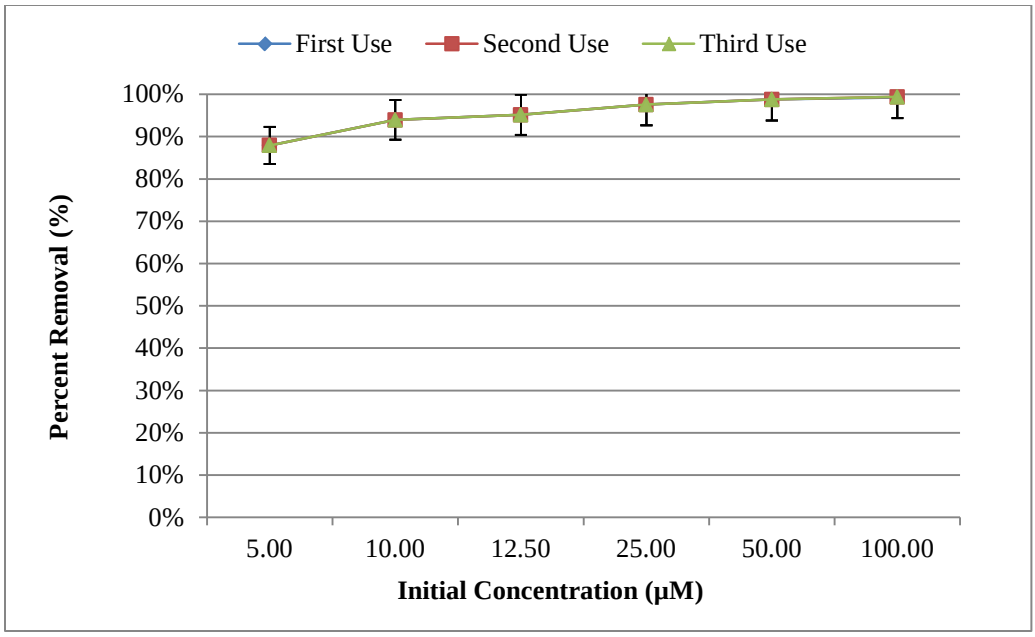

Figure 6. Percent Removal of BPA with Three Rounds of Activated Charcoal Batch Filtration (±2%).

3.6 Effectiveness of Charcoal Chromatography Column Filtration

All filtered concentrations were calculated using the standard curve established in the previous experiment, with the same minimum detectable range and percent error as before. The concentrations

after filtration were typically less than <.604 µM, which is equivalent to a percent removal range of 87% to >99%. One change in percent removal was detected (at the concentration of 12.5 µM between the first and second hours), to be 87% in comparison to 95% in sequential hours. However, this variation may be attributed to measurement error as evidenced by the consistency of the subsequent hours of filtration. The results of the continuous use of the charcoal filter are illustrated by Figures 7 and 8. As an example, Table 3 presents the percentage removal of BPA during the fourth hour of continued filtration.

Paired t-tests performed within all consecutive and continuous filtration resulted in all p-values >>0.05 (95% confidence, α=0.05) which strongly supported the null hypothesis. The null hypothesis was that within the measuring sensitivity and accuracy of spectrophotometer, there is no significant difference in percent removal between consecutive rounds of filtration, nor between continuous hours of filtration.

Table 3. Comparison of Absorbance and Concentration Before and After Chromatography Filtration (after 4 hours)

Initial Concentration (µM)	Initial Absorbance (abs)	Final Absorbance (abs)	Minimum Accurate Absorbency (abs)	Final Concentration (µM)	Removal of BPA (%) ±2%
5	0.117	-0.005	<0.020	<0.604	>88%
10	0.247	0.000	<0.020	<0.604	>94%
12.5	0.367	0.003	<0.020	<0.604	>95%
25	0.612	0.005	<0.020	<0.604	>98%
50	1.135	-0.001	<0.020	<0.604	>99%
100	1.824	-0.001	<0.020	<0.604	>99%

Paired t-tests comparing the percent removal from first, second, and third round of consecutive filtration with that of the one-, two-, three-hour of the continuous filtrations also revealed that all p-values >>0.05 ($\alpha = 0.05$), therefore proving that the continuous filtration is equally effective as consecutive use filtration within the range of the tests conducted in this study.

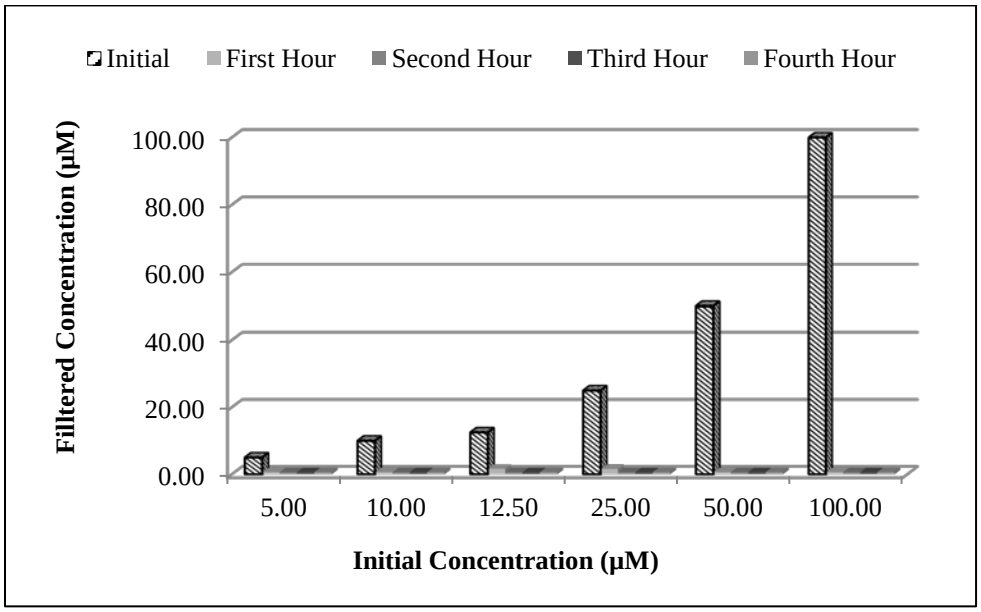

Figure 7. Concentrations of BPA with Four Hours of Continuous Activated Charcoal Chromatography Filtration.

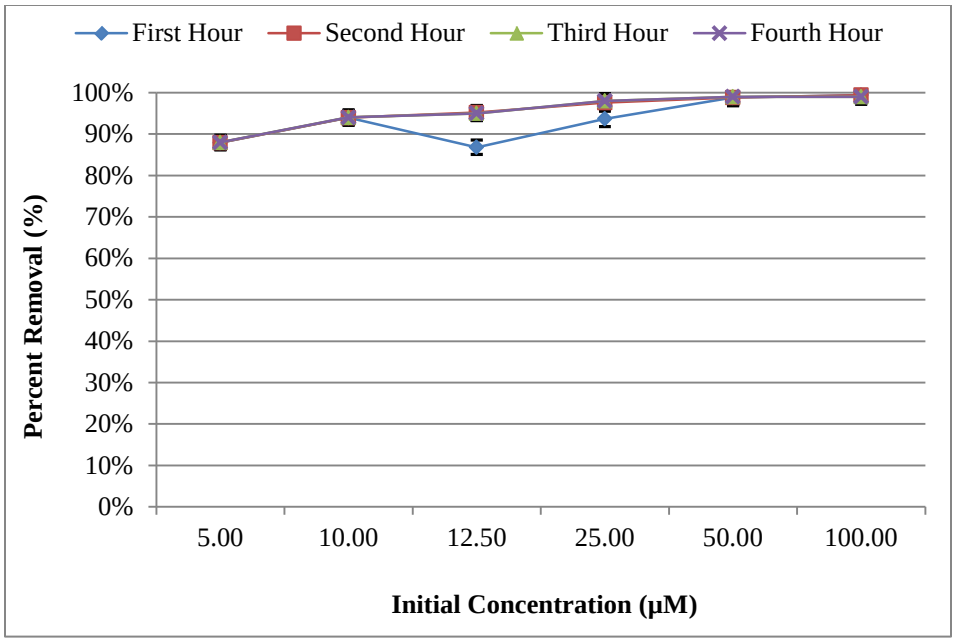

Figure 8. Percent Removal of BPA with Four Hours of Continuous Activated Charcoal Chromatography Filtration (±2%).

4. DISCUSSIONS AND CONCLUSIONS

This study found that activated charcoal can effectively remove BPA from water with an effectiveness as high as > 99%. Even after multiple batches and several hours of use, the saturation point of the activated charcoal was still not achieved, indicating that filters can be reused more than three times or for more than four hours. The activated charcoal filter was also so potent that its capacity for kinetics could not be reliably ascertained with the spectrophotometric assay. Activated charcoal is readily available and economically feasible, and this study provides the theoretical basis for future investigations of activated charcoal's application for filtration of BPA in large-scale municipal water treatment plants, as well as in smaller scale situations of compromised water quality where centralized water treatment is unavailable.

A prior study from Bautista-Toledo et al. (2005) addressed the kinetics of BPA binding to activated charcoal, but did not address the durability or specificity of the response. We find that binding is durable with no loss of effectiveness in multiple re-uses of the activated charcoal. In both multiple use and continuous use experiments with the activated charcoal filter, percent removal of ≥99% BPA at concentrations between 5 µM and 100 µM was achieved. It appears that in both experiments, at lower concentrations, a lower percent removal of BPA is achieved. However, this apparent lower percentage removal could be due to the limitations of spectrophotometer assay. Within the ±2% error, the effectiveness of the charcoal filter did not appear to decrease with multiple uses or continuous use for several hours. We also find that the BPA binding is specific for the activated charcoal as tests done with sand filters, which are currently in place in sewage treatment plants, show that they are largely ineffective at removing

BPA. The apparent increase in absorbance (apparent negative removal) may indicate other contaminants from the sand that can also be detected by the spectrophotometric assay. Sand filtration is not effective at filtering substances like BPA and effectively does nothing to prevent BPA from entering the environment.

The limitation of this study was the sensitivity of the spectrophotometer. Its minimum detectable value is 0.02, which limits the accurate minimum detectable concentration to 0.604 µM. It is highly probable that the actual filtered concentrations of the experiments were below this limit. While these cannot be accurately measured, the data suggests that even with the limited detectability of the spectrophotometer, greater than 87% of all BPA can be removed in all instances of filtration. It is expected that the filtration method used in the second experiment of continuous use of the charcoal filter may actually be less effective than that used in the first experiment because of less thorough incorporation of the filter into the solution, but this also cannot be confirmed due to the limitations of measurement. However, it is highly probable that repeated filtrations with either consecutive use or continuous use of activated charcoal may remove BPA in wastewater to a concentration below the current BPA concentration level in drinking water.

Further research will need to be conducted to make the application of charcoal filters practical on a large scale. The second method of filtration, continuous use, may be promising for its application in consumer uses. Future studies may also include finding more sensitive assays to accurately measure concentrations below 0.604µM so that an accurate percent removal of the BPA levels present in the environment ~500 pM can be ascertained. Additionally, further work will need to be conducted with other types of endocrine disruptors that pose an environmental threat to our waters, such as phthalates, pesticides, and other hormone agonists/antagonists.

5. ACKNOWLEDGEMENTS

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A MORE CONVENTIONAL APPROACH TO ALGAE BIOFUEL PRODUCTION THROUGH INTERNAL ENZYME-MEDIATED CELL LYSIS

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ABSTRACT

Biofuel is viable form of renewable hydrocarbon energy that comes via processing nutrients from biomass sources, existing in the primary forms of biodiesel, biogas, and bioethanol. Bumper crops such as wheat, corn, and soybeans are popular biofuel substrates however algae are currently being investigated as an alternative because they are easier and cheaper to cultivate, and also have higher biomass-to-volume ratios and production rates of lipids and lipid-derivatives. One of the main problems with algae biofuel production is that methods of nutrient degradation and extraction from the biomass are very costly, hazardous, and make biofuel production endeavors unappealing. This experiment analyzes a novel method that uses hydrogen peroxide and the internal enzyme catalase to rupture the phospholipid bilayer, making the extraction of nutrients safer, more cost-effective, and environmentally-favorable. The algal strains tested were *Ankistrodesmus falcatus, Scenedesmus quadricauda, Nannochloropsis oculata*, and the P_S wild-type isolated strain. Microscopy, spectrophotometry, and both batch and micro-assays confirmed that the novel lysis method was successful in vitro within all algal strains. Post-experimental data analysis led to the formation of a theoretical bioprocess system, as well as real-world applications for this method.

Keywords: Biofuel, algae, molecular biology, spectrophotometry, biolipids, metabolic pathway, bioreactor

1. INTRODUCTION

The current nature of biofuel production utilizes lipids and carbohydrates from bumper crops such as wheat, corn, and soybeans, which are processed through the enzymatic, catabolic, or mechanical destruction of cells, subsequently releasing nutrients ([Pimentel and Patzek, 2005], [McPhail and Babcock, 2008]). However, the use of bumper crops was deemed inefficient as the growth demands far surpass the profitable nutrient potential; they require significant amounts of land, clean water, and sufficient nutrient supplementation ([Hannon *et al.*, 2010], [Onuki, n.d.], [Jeon *et al.*, 2013], [Sander and Murthy, 2009]). The ineffective fuel-yield of bumper crops as biofuel substrates was represented by McPhail and Babcock (2008), using bioethanol as a model.

I. Standard corn cultivation and ethanol production capacity [a]:

$$Q_{c,t}^{D,ethanol} = \lambda_t \cdot \tilde{E} \cdot \theta_t$$

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II. Planted acreage probability distribution of yield per harvested acre (in bushels) for 2008 [b]:

$$\tilde{y}_{c,2008} \sim beta(\tilde{y}, \sigma_y^2, p_y, q_y)$$

According to their models from 2008 and 2009, approximately 2.75 gallons of ethanol was produced for each bushel of corn at full functioning distillation plants. Calculations revealed that the relatively high price of $4.97 per bushel (2008/2009 values), The Energy and Independency and Security Act (EISA) mandates, and the relatively high price of taxes ($0.51-per-gallon blenders tax) spurred low ethanol yields for 2008 (approximately 500 million gallons below standard EISA mandates). Statistical analysis shows these speculations to be steadfast as the high price of fossil fuels, governmental mandates, and economic trends work against farms. This data illustrates the current economic challenges that farmers face, in addition to high maintenance costs in the use of bumper crops for inefficient bioethanol production (McPhail and Babcock, 2008).

Microalgae are currently under intensive investigation because they are extremely easy to cultivate, and are less costly due to lower nutrient and volume requirements; their growth biomass-to-volume ratio is much higher than that of bumper crops ([Gao *et al.*, 2012], [Hannon *et al.*, 2010], [Sudhakar and Premalatha, 2012], [Oilgae, 2013], [Luo *et al.*, 2010], [Elakkanai, 2011], [*Algae Biomass Organization*, 2013], [*Algae University*, 2014]). On average, algal cultures can produce as much as 5,000 gallons of biofuels per acre, per year, and are more desirable because they do not have to compete with other crops for land. This hearty nature allows the cultivation of algae to occur on more confined stretches of land (Laing, 1991). Algae also produce, on average, more glycogen and lipids than conventional crops (Gao *et al.*, 2012) [Fig. 1]. Following carbon sequestration, two metabolic pathways converge which direct the carbon products of photosynthesis into either lipid synthesis, directed by the enzyme *Acetyl CoA Carboxylase*, or complex carbohydrate synthesis, completed by dehydration synthesis of monosaccharides into polysaccharaides (Gao *et al.*, 2012).

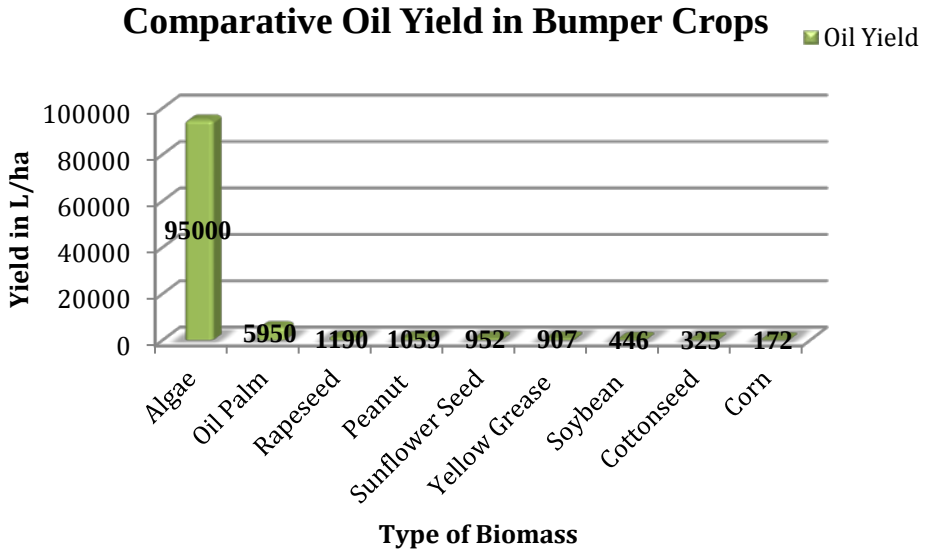


Figure 1. Triglyceride count in liters per hectare for varying bumper crops (Gao et al., 2012).

1.1 Current Production Problems:

1.1.1 General Production Methodology

In most scenarios, the simple decay of biolipids involves the harvesting of algae, biomass processing, and then finally oil extraction when the algae has formed a "cake" at approximately 15-25% total suspended solids ([Oilgae, 2013], [Arthur and De Flamingh, 2011]). The subsequent oil extraction leaves behind a slurry of nutrients high in starches, carbohydrates, and saccharides. These sugars, after hydrolysis of complex carbohydrates, can provide a substrate for ethyl alcohol fermentation through the use of *Saccharomyces* and anaerobic respiration, which produces ethanol through the reduction of pyruvate ([Onuki, n.d.], [Luo *et al*., 2010]). Lipids harvested from the algae biomass can precipitate out of solution and can then be used for the creation of biodiesel, heating oil, and other subsequent low octane fuels. Before the desired product can be used, the glycerol groups must be removed from the triglyceride lipids by a process known as transesterification. For this, an alcohol such as methanol or ethanol is reacted with the triglycerides and a catalyst, which is usually sodium hydroxide or potassium hydroxide, however, the latter is used more commonly used because of its increased strength. Under heat, the reaction separates the glycerol from the triglyceride producing methyl fatty acid esters, or ethyl fatty acid esters, respectively. This method has 98% efficiency when it comes to the creation of biofuels, however, producers need to be wary of any water during transesterification, as this will lead to saponification. ([Ho *et al*., 2010], [*OriginOil*, 2010], [Mandjiny *et al*., 2006-2007]). Washing the biolipids prior to transesterification is often recommended, as any water present will lead to the creation of soap and glycerin from triglycerides, preventing the full conversion of raw lipids into free fatty acids (saponification). Once lipids are extracted from the lysed biomass, it is very simple to create low octane bio-oils from either this chemical process, or simply letting the lipids naturally decay over an extended period ([Gao *et al*., 2012], [*Algae Biomass Organization*, 2013], [Ho *et al*., 2010], [*OriginOil*, 2010], [Mandjiny *et al*., 2006-2007]).

1.1.2 Inefficient Photobioreactor Cultivation Methods and Yields

One of the main problems in algae biofuel production actually lies in the nature of the biomass-to-volume ratio. Although they are among the fastest growing plantae organisms, and can potentially contain a dry mass of 50% oil, a significant amount of biomass needs to be cultured in order to produce lipid and carbohydrate yields that are suitable to meet national and industry standards. With current technologies, it is estimated that the price per barrel of algae based petroleum would cost anywhere from $300 - $2600 U.S. dollars; such a value is exponentially higher than current petrol prices per barrel, estimated at $97.49 [WTI Crude Oil], and $106.40 [Brent Crude Oil], both as of February 1st, 2014 (Hannon *et al.*, 2010). A variety of factors, such as weak harvesting methods, purification technologies, and transport systems constitute some of this problem, yet a significant portion relates to the necessity of obtaining enough biomass so that a profit can be made and funds are not lost during culturing. This notion was explained by Arthur and De Flamingh (2011), who used the term Net Energy Ratio (NER) to explain the return-investment of algal mass-culture. An NER of 1 represents homeostasis between the amount of energy put into culturing and the amount of energy within the subsequent biomass

(Ho *et al*., 2010). Weissman's report discussed certain growing challenges, stating that closed vs. open system bioreactors need to be investigated, as well as the fact that growing conditions vary for different algal strains, and both temperature and light intensity variation could produce different results in different reactors (Ho *et al*., 2010). Algae biofuel production is still in its infancy, and although researchers have proven them to be ideal organisms because of high lipid content relative to volume, culturing methods still need to be refined to perfect the most efficient conditions for strain isolation and increased biomass production.

1.1.3 Costly, Inefficient, and Non-Universal Lysis Techniques

Currently, the development of algal bioreactors is relatively young and therefore they have not reached an industry-viable state yet. The main part of this problem derives from inefficient lysis extraction methods and the actual components that go into the creation of a photobioreactor. The main methods for the extraction of desired products from the algae involves both solvent and ultrasonication lysis ([Oilgae, 2013], [*Algae Biomass Organization*, 2013], [OriginOil, 2010]). In the former, solvents are used to lyse cells, while the latter uses the generation of high frequency waves to homogenize the cells in order to extract nutrients to produce fuel. Generally, the solvent method is not desirable because of the cost and hazards involved. It is inefficient for large industries because of the slow-batch process used with solvents and because of the high cost of large quantities of organic solvents ([Gao *et al.*, 2012], [Hannon *et al*., 2010]). Ultrasonication is not an option for small-scale producers because of the expensive costs required to purchase and maintain a sonication device. Realistically, this method is the most efficient for large-scale industrial biofuel producers but nevertheless it is still very costly and there are problems with vibrating machinery, power-input, and the complications that arise from its use in both flow and batch-style reactors ([Gao *et al.*, 2012], [Hannon *et al*., 2010]). A third style that is employed is the standard cake formation method, in which the supernatant is filtered off and the algae biomass is manually manipulated to release both the carbohydrates and lipids (Oilgae, 2013). This method is inexpensive yet inefficient, as it is time consuming and has relatively low yield rates. Enzymatic cell lysis is an alternative to all these methods and uses degradarion enzymes such as pectases, amylases, and lipases to destroy both the cell wall and phospholipid bilayer (Sander and Murthy, 2009). Enzymatic lysis is very precise as titration techniques can be employed allowing producers to add the exact amount of enzymes that need to be used. It can also be implemented in "flow-style" reactors and avoids the time spent waiting for algae cake formation and degradation. However, enzymatic lysis is undesirable as enzymes needed are often expensive and difficult to isolate (Gao *et al*., 2012).

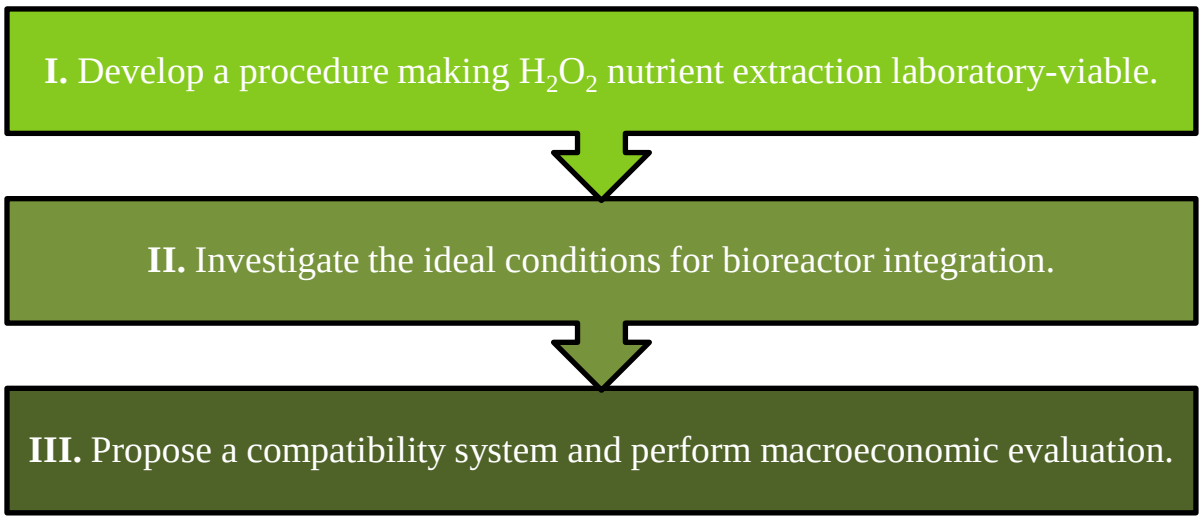
2. EXPERIMENTAL OBJECTIVES

Enzymatic cell lysis is an alternative method used in nutrient extraction. In this experiment, I revisit "enzymatic" degradation in a new light by utilizing the function of catalase, an internal enzyme that facilitates the decomposition of H_2O_2 in vivo [Fig. 2]. Through the rapid buildup of gas pressure and the toxicity of the peroxide, the theoretical lysing of the algal cells should occur. Due to the tough nature of the cell wall, it is speculated that it will have to be destroyed before catalase-based degradation can be effective, either through cheap extracellular fermentative enzymes (amylase and pectinase) or induced plasmolysis (through the addition of salts such as KCl and NaCl).

$$2\left(H_2O_2\right) \xrightarrow{Catalase} 2\left(H_2O\right) + O_2$$

Figure 2. The decomposition of hydrogen peroxide mediated by catalase.

2.1 Experimental Goals and General Overview



3. METHODS AND MATERIALS

3.1 Strain Selection and Cultivation Methods

Algae selected for this experiment were based on relatively high lipid yields and include *Ankistrodesmus falcatus* and *Scenedesmus quadricauda* [both purchased from Carolina Biological Supply Company, Burlington, NC], as well as *Nannochloropsis oculata* [purchased from Algabiotic Research Technologies, Inc., McKinney, TX] [Fig. 3]. A fourth wild-strain pond algae (P_S) was used for comparative testing, as well as results that would reflect the possibility of micro-harvesting and culturing by individual producers from local sources (in natura); the strain was created through isolative subculturing over the course of eight weeks. This involved screening to remove foreign objects and organisms, and selective subculturing. This strain of algae was isolated in situ from a non-stagnant koi pond at Columbia Senior High School, 17 Parker Ave., Maplewood, New Jersey, 07040.

Microalgal Species (freshwater)	**Lipid Content (based on % of dry weight)**
Nannochloropsis	46 %
Ankistrodesmus	28-40 %
Scenedesmus	45 %
P_S Wild-Strain Algae	*Not Applicable*

Figure 3. Chart depicting the algal strains selected for investigation. Data based on information from Oilgae (2013).

Algae cultures were grown in a variety of different vessels, the primary ones being erlenmeyer flasks and sterilized 500 ml plastic water bottles. The algae was cultured in 22^o C conditions, with a pH of about 7.8 for all cultures, and grown on modified Allen Media (referred to as AM_Y henceforth) with small amounts of a 0.2 molar HCl solution (Allen Medium, *Cyanosite*, n.d.). Growing stock cultures of algae primarily utilized 75% distilled water and 25% AM_Y media, respective to the size of the growing vessel. Stock AM_Y media was made with a base of 1000 ml of pure distilled H_2O and with the following solutes and concentrations: $NaNO_3$ [1.5 g], K_2HPO_4 $7H_2O$ [5 ml of a 6 g/L solution], $MgSO_4$ $7H_2O$ [5 ml of a 6 g/L solution], Na_2CO_3 [5 ml of a 4 g/L solution], $CaCl_2$ $2H_2O$ [5 ml of a 2.5 g/L solution], Citric Acid - H_2O [0.0048 g], and a modified P-IV metal solution containing: $MgCl_2$ [2 ml of a 99.7 % solution], NO_3 [0.0003 %], PO_4 [0.0002 %], SO_4 [0.0005 %], Ba [0.002 %], Ca [0.004 %], Fe [0.0001 %], Mn [0.0002 %], and St [0.001 %] – the P - IV metal solution had a pH of 5.5, assayed by A.C.S spectrometer standards. A pH of 7.8 was recorded and culture media was stored in 1000 ml erlenmeyer flasks at a temperature of 4^o C. until time of culturing. 30% aeration was given to the cultures on a 12 hr cycle, and cultures were grown under 40 watt aquarium lights on a 12 hr dark, 12 hr light cycle [Fig. 4].

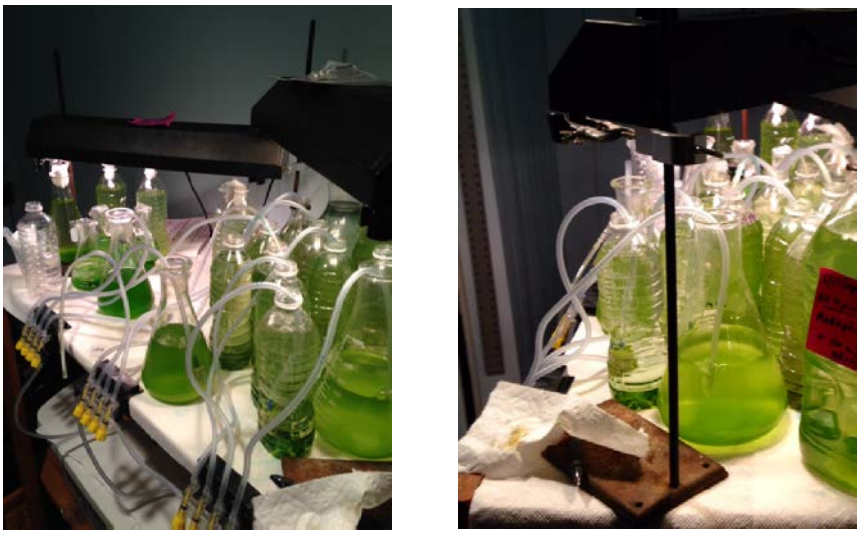


Figure 4. Images of the home laboratory in which the algae was cultured.

3.2 Catalase Function Optimization

The enzyme catalase was investigated in a series of different organisms to map its efficiency and compare the optimal functioning results. This investigation was conducted on yeast and P_S algae, as well as N. oculata, A. falcatus, and S. quadricauda in select conditions. It was conducted by utilizing filter paper disks [Fisherbrand Qualitative Filter Paper] with a radius of 0.5 cm, which were either soaked in a yeast solution or soaked in an algae solution and then dried. By measuring the time it took the H_2O_2 to decompose as a result of catalase, or the time it took the disks to rise to the surface, the effectiveness of the catalase could subsequently be mapped (College Board, 2012). The test conditions constituted a normal temperature of 20^o C, a temperature of 50^o C, a basic environment of KOH at a pH of 10, an acidic environment of HCl at a pH of 3, and then a neutral pH control; in these types of tests, a 15% H_2O_2 solution was used. Concentrations of H_2O_2 were also tested in a neutral, room temperature environment and included 100%, 50%, and 25% treatments of a 30% H_2O_2 solution, and 100%, 50%, and 25% treatments of a 3% H_2O_2 solution. Test canisters contained a volume of 30 ml. The yeast culture contained a density of 0.061 g/ml, while the algae cultures contained a density of 0.043 g/ml. In the algae experiment, 1 ml of dry amylase was added to the cultures for 5 minutes of incubation prior to the creation of the filter disks.

3.3 Measurement Equipment

All advanced measurement tools, such as pH meters, dissolved oxygen sensors, etc. were purchased from Vernier Scientific. A USB Red Tide Vernier Spectrophotometer was used in all pigment and quantified growth assays. Vernier Logger Pro. was used in the extrapolation and analysis of all data, as well as a handheld Vernier LabQuest for the collection of data.

3.4 Pigment Absorption Assays for Quantifying the Novel Lysate Method

The novel lysis technique using catalase was investigated for efficiency by the amount of free chlorophyll that was detected in the supernatant treatment solutions. Pigment values in the solution were assayed using a spectrophotometer and then analyzed using the pigment absorption equations developed by Arnon (1949), Lichtenthaler and Wellburn (1983), and Porra (2002). However, values from the spectrophotometric assays were ultimately analyzed solely using Porra (2002) chlorophyll extraction equations as it was proven by Porra that his equations were more efficient quantifiers than the Lichtenthaler et al. and Arnon equations [Table 1]. The subsequent values were used as an indicator of the effectiveness of cell degradation. Algal cultures were suspended in their respective treatments for a 24 hr period and then assayed. Normalized pigment extractions were calculated for all four strains by submerging the algae in 90% acetone to extract chlorophyll a, b, and other miscellaneous carotenoids and xanthophylls. Plasmolysis was also a variable tested in this experiment, which theoretically, in combination with both an enzyme and peroxide treatment, could make cell lysis more effective. Enzyme treatments consisted of incubating the algae cultures with amylase and pectinase, 10 ml. of each at an optimal density of 0.5 g/ml. They were incubated in a 30^o C water bath for 30 minutes prior to treatment. For the plasmolysed solutions, the cultures were incubated in a 5 M NaCl salt solution for 10 minutes before and after H_2O_2 treatment for comparative testing. Peroxide values used were 15% H_2O_2 (~ 50%, 30% H_2O_2) due to the results of Section 4.1 for all solutions. The magnesium and calcium solutions that were used on certain algae treatments were both at 0.2 M

- these ions were tested because of their role in common lysis buffers (Tsugama *et al*., 2011). In Section 4.3, Mg^{2+} and Ca^{2+} treatments are denoted by Ψ, while control conditions are denoted by Λ.

3.5 Assay Overview

Three assays were ultimately conducted for this experiment: (a) an assay investigating plasmolysis and enzyme treatment in combination with H_2O_2, (b) an assay investigating different concentrations of H_2O_2, and (c) an assay investigating the ideal order that the lysate treatment should follow. In assay (c), Mg^{2+} and Ca^{2+} were also tested for their role as common lysis buffers.

Table 1. Arnon (1949), Lichtenthaler and Wellburn (1983), and Porra (2002) chlorophyll extraction absorbance equations. Porra's equations were determined to be the most accurate (to date) and were exclusively used.

Arnon Equations:

Total chlorophyll (μg/ml) = $20.2(A_{645}) + 8.02(A_{663})$

Chlorophyll a. (μg/ml) = $12.7(A_{663}) - 2.69(A_{645})$

Chlorophyll b. (μg/ml) = $22.9(A_{645}) - 4.86(A_{663})$

Lichtenthaler et al. Equations:

Chlorophyll a. (μg/ml) = $12.21(A_{663}) - 2.81(A_{646})$

Chlorophyll b. (μg/ml) = $20.13(A_{646}) - 5.03(A_{663})$

Carotenoids (μg/ml) = $\frac{(1000A_{470} - 3.27[chl(a)] - 104[chl(b)])}{277}$

Porra Equations:

Total chlorophyll (μg/ml) = $17.76(A_{646.6}) + 7.34(A_{663.6})$

Chlorophyll a. (μg/ml) = $12.25(A_{663.6}) - 2.55(A_{646.6})$

Chlorophyll b. (μg/ml) = $20.31(A_{646.6}) - 4.91(A_{663.6})$

4. RESULTS

4.1 Modeling Catalase Function *In Vivo*

Results concluded that in active dry yeast cultures, warmer conditions with a higher concentration of peroxide reacted the fastest. A direct correlation was seen between the function of catalase and concentration of peroxide- the higher the concentration, the faster the decomposition reaction occurred [Fig. 5].

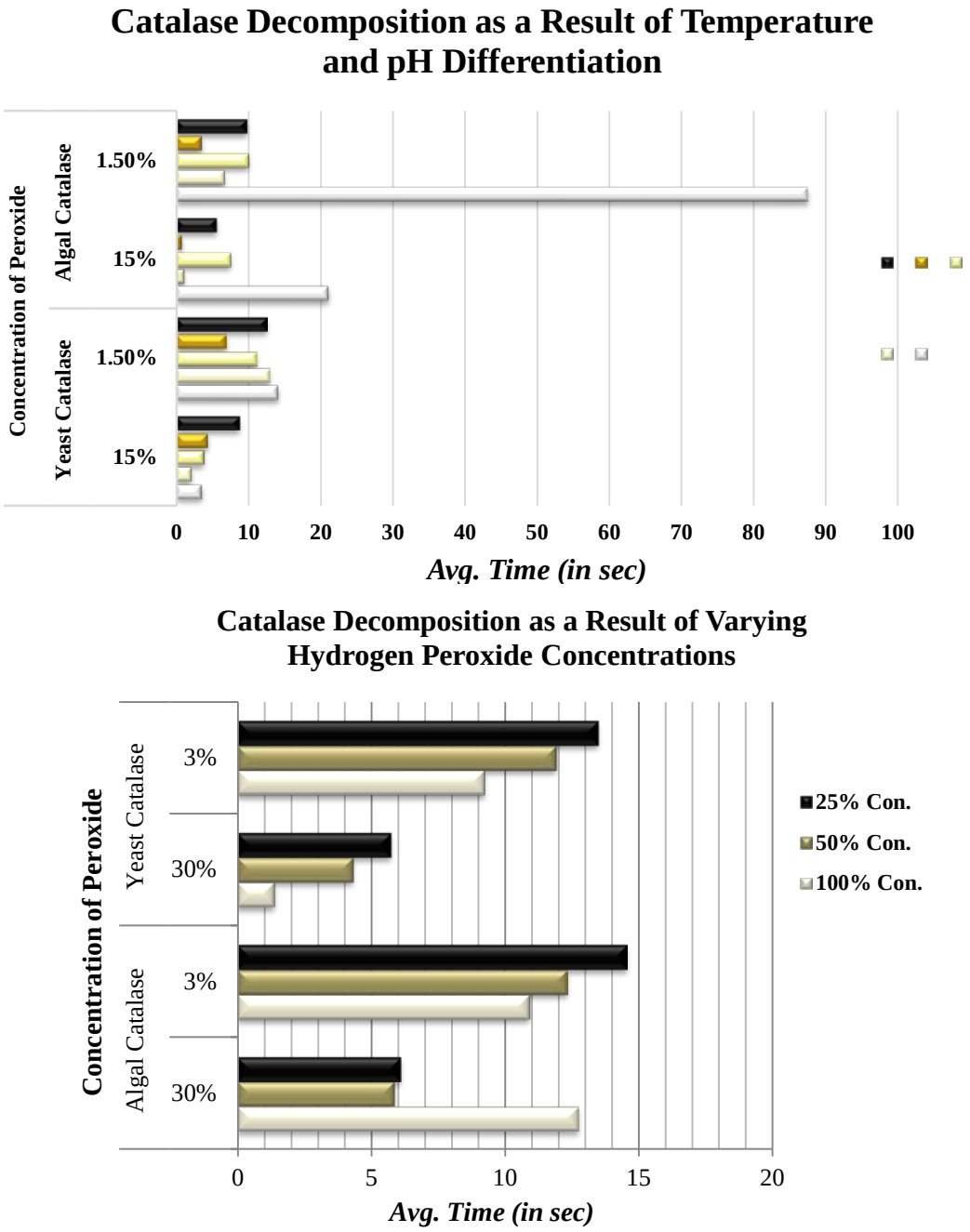


Figure 5. Data mapping the optimal conditions for rapid hydrogen peroxide decomposition. The upper graph depicts pH differentiation on catalase functioning while the bottom depicts varying H_2O_2 concentration on catalase functioning.

In P_S algae, basicity produced very rapid decomposition rates while acidity drastically slowed down the function of catalase. Examining concentration alone, a direct correlation was seen between the concentration of H_2O_2 and the time of the reaction, except in pure 30% H_2O_2. Optimal concentration for catalase functioning in the P_S strain was valued at 15% H_2O_2 (solely through examining the internal enzyme), for at higher concentrations of peroxide the reaction took much longer, and in some cases went on indefinitely, suggesting enzyme denaturation or inhibition [Fig. 5].

4.2 Cell Wall Degradation

Benedict's test confirmed that amylase and pectinase are effective in degrading the algal cell walls as indicated by copper (II) oxide precipitation using Benedict's assay [Fig. 7], however no significant value of reducing sugars was detected in batch supernatant testing. Microscopy reveled that plasmolysis is also effective in cell wall degradation and post-analysis confirmed that the latter method is more efficient [Fig. 6].

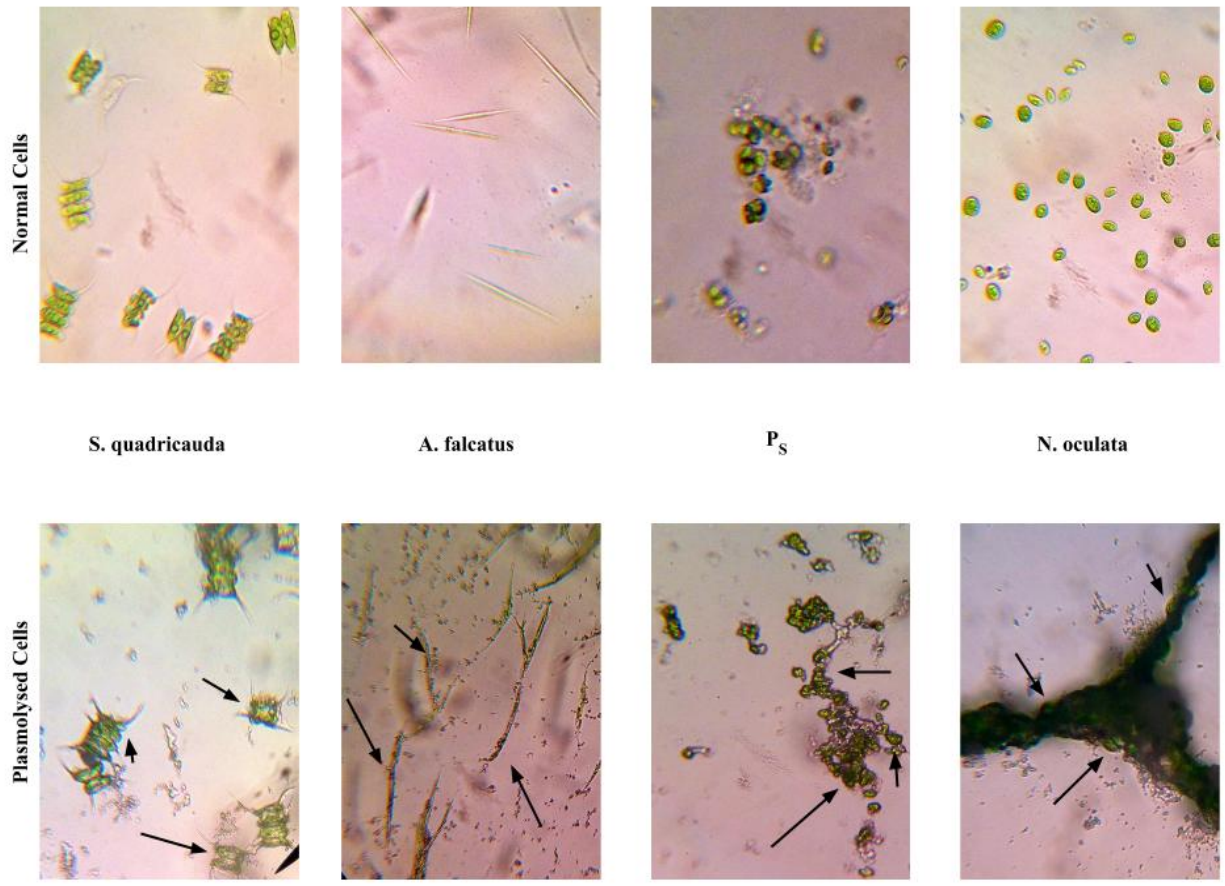


Figure 6. Microscopy revealing plasmolysed algal cells. The arrows indicate areas where clear deformities and cell wall damage is present.

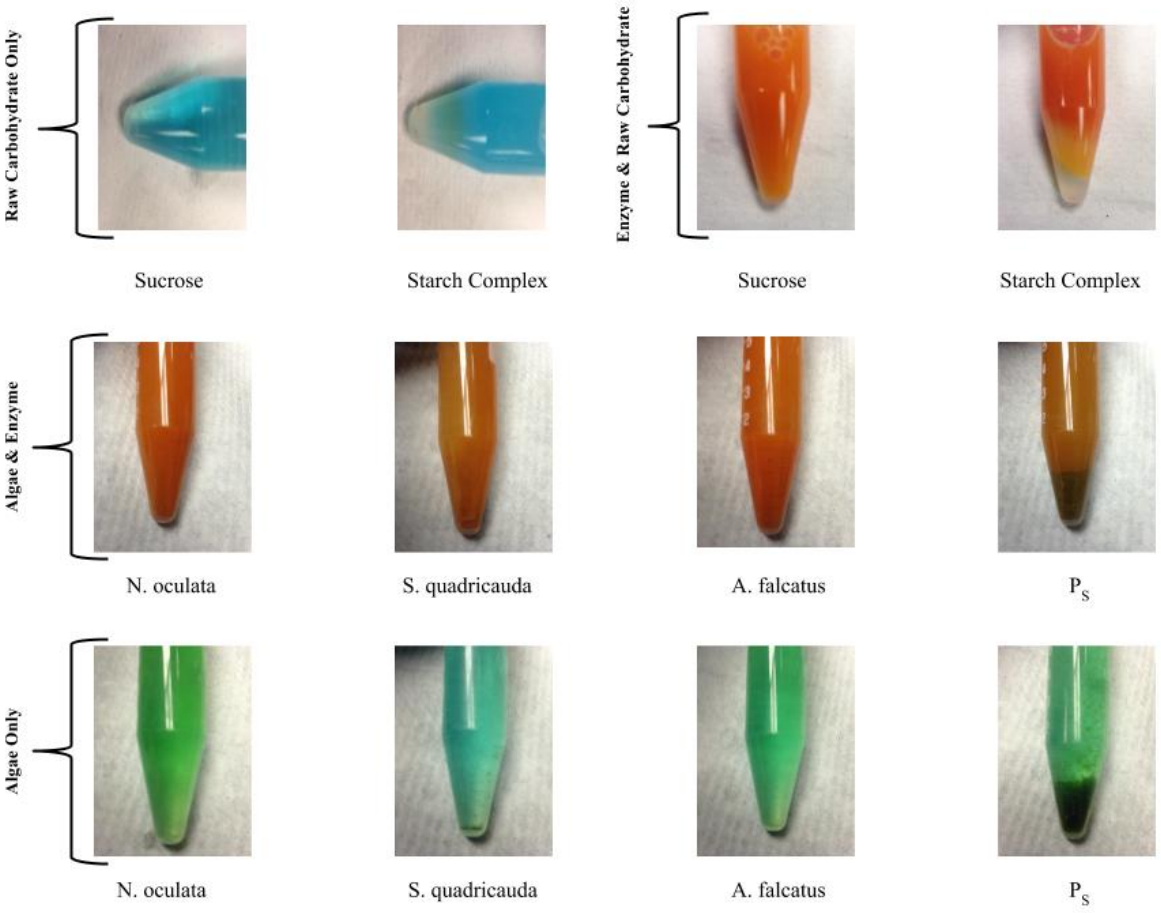


Figure 7. Benedict's reagent test confirming that amylase and pectinase are effective in cell wall degradation.

4.3 Spectrophotometric Quantification of Degradation

Spectrophotometry and pigment assays revealed that the plasmolysed, enzyme-treated solutions were the most successful in inducing cell degradation [Fig. 10]. The concentration assay provided data that suggested that each algal strain possesses a different H_2O_2 concentration ideal for degradation [Fig. 9]. However, 15% was determined the optimal universal concentration. Each strain preferred different times for the addition of reduction enzymes [Fig. 8].

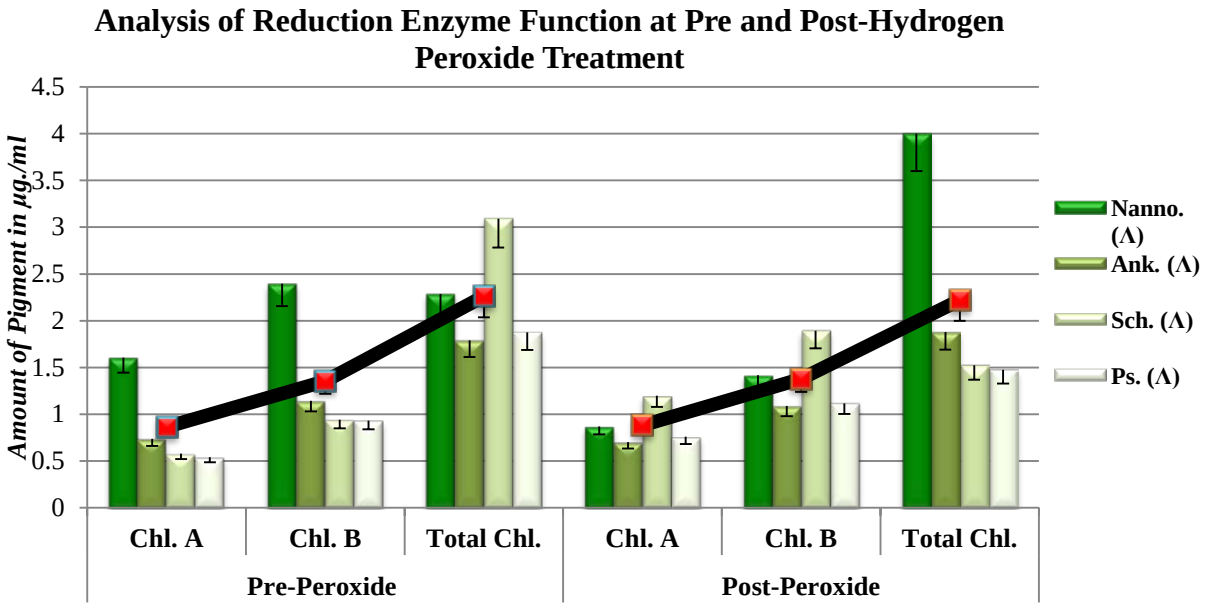


Figure 8. Bioprocess assay analyzing the points where amylase and pectinase are more effective in contributing to cell degradation. 15% H_2O_2 was used for treatments. Λ indicates no buffers were used.

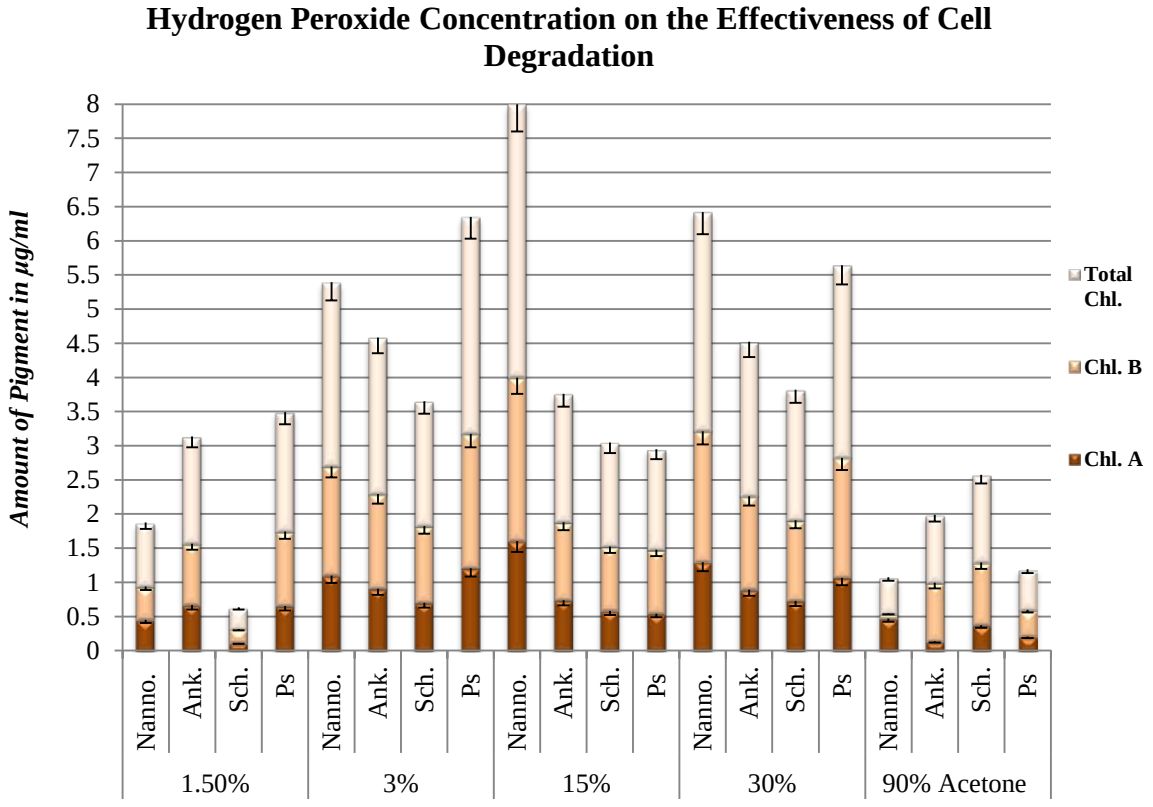


Figure 9. Assay investigating varying hydrogen peroxide concentrations in relation to cell degradation.

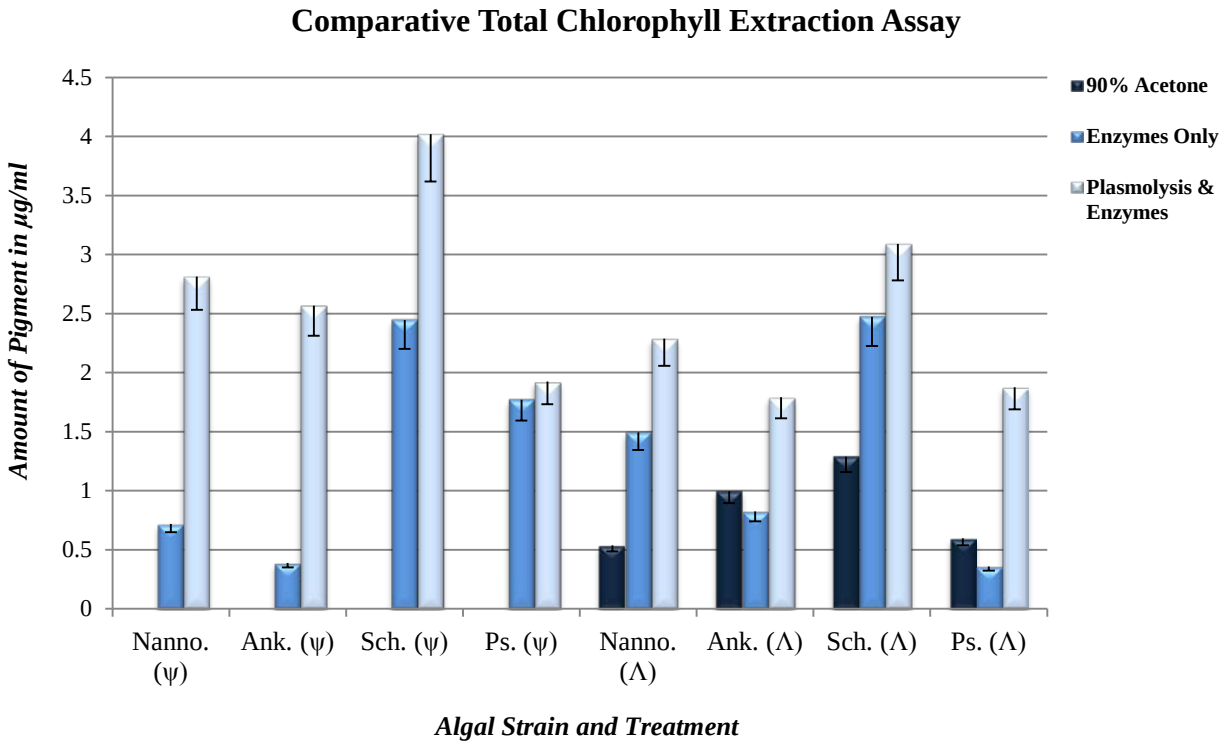


Figure 10. Assay investigating different treatments and their overall effectiveness in relation to cell degradation. 15% H_2O_2 was used for treatments. Ψ indicates Mg and Ca buffer compounds were used. Λ indicates no buffers.

4.4 Macro-batch Analysis

Qualitative testing revealed that extractable lipids were present in the algal supernatant treatment solutions, however, minimal sugars were detected in the supernatant solutions. The toxicity of the H_2O_2 makes processing the lysate difficult (yeast cannot ferment and lipid harvesting and/or transesterification may be inhibited). $NaHCO_3$ and Na_2CO_3 were discovered as inhibiting compounds, resulting in a negative catalase reaction from yeast in post-lysis solutions [Fig. 11]. Combining hydrogen peroxide with sodium bicarbonate and sodium carbonate yields the following products:

(i) Sodium carbonate reacts with hydrogen peroxide to form either carbonate peroxyhydrate or peroxycarbonate (Lee *et al*., 2000).

(ii) Sodium biocarbonate reacts with hydrogen peroxide to form peroxobicarbonate. Peroxobicarbonate then reacts intramolecularly to form carbon dioxoide and sodium percarbonate (Firsova *et al*., 1968).

(i) $Na_2CO_3 + H_2O_2 \rightarrow Na_2CO_3 \bullet H_2O_2$
$Na_2CO_3 + H_2O_2 \rightarrow Na_2CO_4 \bullet H_2O$

(ii) $NaHCO_3 + H_2O_2 \rightarrow NaHCO_4 \bullet H_2O$
$NaHCO_4 \bullet H_2O \rightarrow CO_2 + Na_2CO_3 \bullet 1.5\ H_2O_2$
(unbalanced)

These compounds, although unstable, have been shown to rapidly accelerate peroxide decomposition and therefore can easily neutralize a solution of excess H_2O_2. With bicarbonate's reaction to peroxide, a peculiar compound is created (sodium percarbonate), which is explained by Firsova *et al*. (1968). Although only preliminary tests were conducted, these compounds could prove useful in the further processing of biolipids for fuel production.

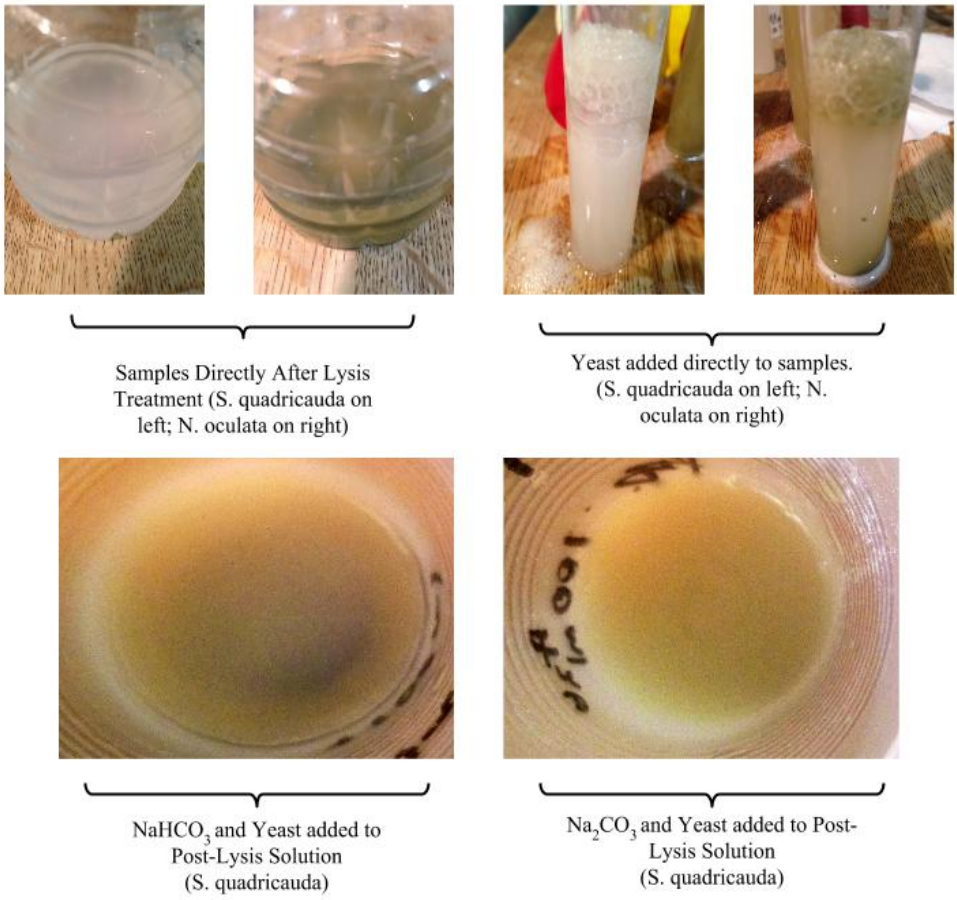


Figure 11. This image depicts the preliminary investigation of neutralization compounds to remove excess H_2O_2 from solution. In the upper-right you can see a (+) catalase reaction with just yeast and the lysate solutions. On the bottom, in the carbonate and bicarbonate lysate solutions, a (–) yeast catalase reaction occurred.

5. DISCUSSION

Analysis of data led to the following proposed treatment integration system that utilizes closed-system bioprocessing modules to create synergy and symbiosis between H_2O_2 production, waste water treatment, and algae biofuel production [Fig. 12]. This method does require more economic investigation to determine its feasibility on the macro-level, however, it offers an alternative method to biomass degradation that could be safer, cleaner, and more cost-effective. Ultimately, the following outcomes were successful of this experiment: (a) the proposed novel lysis and degradation method was successful in vitro in all algal strains, (b) the evaluation of

other methods of nutrient extraction provided evidence that this method could be an alternative to solvents and ultrasonication for it is cleaner, safer, environmentally-friendly, and more feasible for small-scale and individual producers or investors, (c) the proposed macro-model integration bioprocessing system creates a closed-series biofuel processing plant that can be implemented closer to urban areas because of low hazards and limited pollutant factors, enabling in situ production of fuel and lowering plant-to-distributor costs, and (d) the proposed macro-model creates a system which could allow for mass-production of domestic based hydrocarbons from sequestered carbon dioxide, as domestic production of fuel is a crucial step towards economic rebound, energy-independence, and increased green energy systems; it specifically serves as a model for developing nations as well.

5.1 Novel Lysis Method – Future Possibilities?

Data analysis concluded that using catalase and H_2O_2 as a method of cell lysis and nutrient extraction was effective only when plasmolysis and subsequent cytorrhysis was induced beforehand. The extracellular enzymes amylase and pectinase were revaluated for their roles in this process and I discovered that they are more effective in contributing to nutrient extraction and decay if they are added after the H_2O_2. Plasmolysis and peroxide causes cell lysis, where these enzymes can then be added to reduce complex starches and free lipids from the biomass through the reduction of sugars. Lipids are either bound to the central vacuole as triglycerides, or stored in the cell membrane as glycolipids and phospholipids (Schideman, 2014). This method is still in its infancy, but it has many different future applications. It is more desirable for small and individual producers because it avoids the use of hazardous solvents and expensive ultrasonication methods, however, it is important to note that in high concentrations, H_2O_2 can be harmful because it is a powerful oxidizer and the industrial production process of H_2O_2 can be costly. This creation process revolves around the treatment of anthraquinone, and it is relatively efficient as the anthraquinone can be completely recycled. However, it does require a steady supply of H_2 and electricity to run the mechanisms, yet one can use renewable electricity or biogas for producing H_2 (“Hydrogen Peroxide,” 2014). This method of nutrient extraction can easily be employed in parallel with water treatment plants, biomass culturing plants, power plants, and biogas, biodiesel, and bioethanol production centers [Fig. 12.]. Such models can be employed in smaller spaces and closer to dense populations as there is less danger and no exhaust sources, as everything is theoretically recycled.

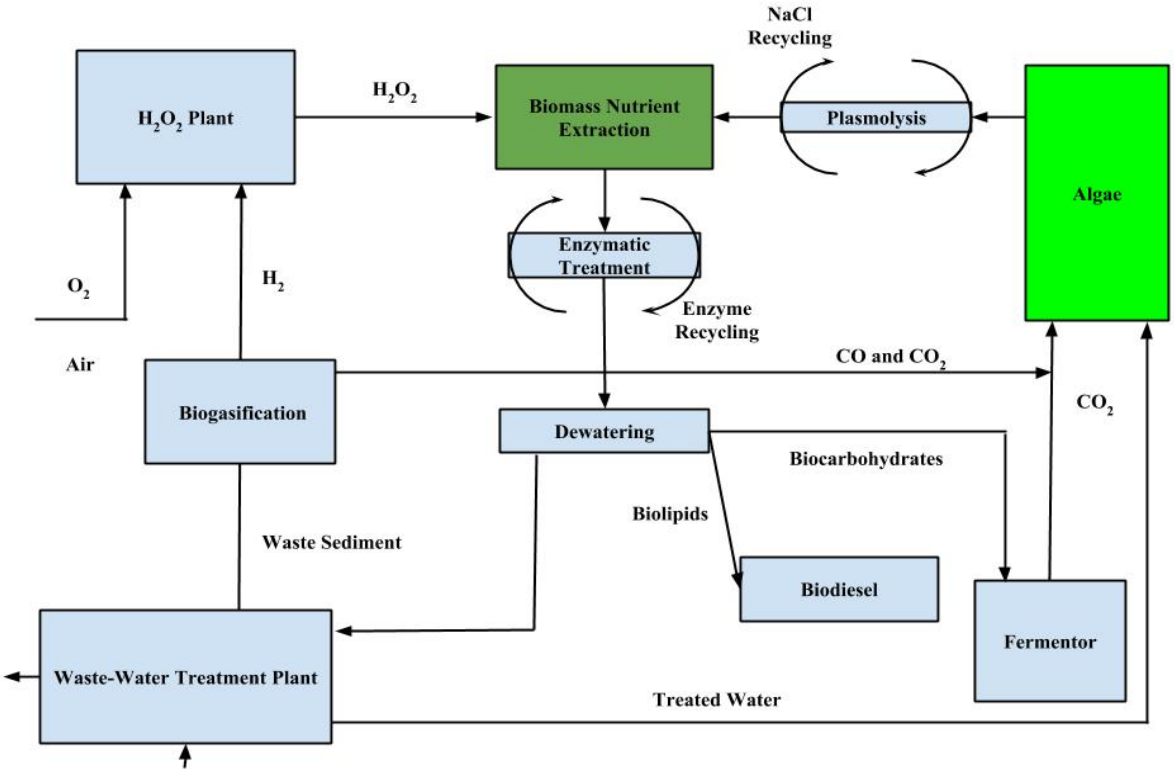


Figure 12. Theoretical model of an ideal, parallel system that creates a self-sustaining method of water treatment, biodiesel, biogas, and bioethanol processing. This system would produce enough fuel to sustain itself as well as excess for the surrounding economy.

This method of nutrient extraction for biofuel production requires further research to investigate the possibility of conservation and filtration mechanisms, which may allow for the recycling of salts, extracellular enzymes, and the prolonged use of H_2O_2. This method could be a very promising way to extract nutrients from biomass, making production more appealing and efficient to small-scale and individual producers, as well as the developing economies of the third-world.

6. CONCLUSION

This research on a novel method of lysis and nutrient extraction using H_2O_2 and internal catalase proved to be very successful when plasmolysis was induced beforehand. This method can potentially be introduced into a continuous flow biomass processing system, which will avoid the use of batch reactors in the harvesting of biomass (Gao *et al*., 2012). By doing so, time and resources can be saved while also employing a method that is more cost effective, safer, and environmentally friendly. This proposed system [Fig. 12] allows nations to integrate clean energy while maintaining a completely self-sufficient fuel production method.

Going forward, future studies should be aimed at conducting micro-lipid and micro-carbohydrate assays to better quantify the results as an alternative to the spectrophotometric assays. Additionally, more extensive research needs to be conducted on the processing of the lysate fluid, which seems to be a difficult challenge. Reactor prototypes and micro-model designs

should be drafted, and the proposed system in Figure 12 should be subjected to a more extensive economic evaluation as well. The goal should be to achieve closed-state symbiosis in the laboratory before further scaled tests could be conducted.

7. ACKNOWLEDGEMENTS

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REMEDIATION OF PCB CONTAMINATED SOILS THROUGH nZVI INTEGRATED SOIL WASHING

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ABSTRACT

Polychlorinated biphenyls (PCBs) have been identified as environmental hazards for years. Due to historical issues, a considerable amount of PCBs have been released into deep underground. In this research, a nanoscale zero-valent iron (nZVI) aided dechlorination, followed by soil washing treatment, was processed to remove PCBs from soil. An artificial contaminated soil was prepared to conduct the experiments. In nZVI aided dechlorination, the effects of the nZVI dose and initial pH level were evaluated. Results indicated that PCB congeners can be effectively dechlorinated. PCBs with lower chlorine atoms in the structures have relatively higher dechlorination rates in comparison with the ones containing more chlorine atoms, showing that lower chlorinated biphenyls are easier to be dechlorinated than higher chlorinated biphenyls. The selected dosage of nZVI was 7.5 g/kg soil, with the removal rates of Tetra-15.6 and Tetra-16.3 around 75% and 65%, respectively. When the pH level in soil is lower than 5, the pH change does not influence the removal rates of PCBs very much, indicating the presence of sufficient protons in the system. Ferric oxides formed during dechlorination were capable of enhancing PCB removal by soil washing since the interfacial tension between PCB droplets and the soil could be reduced by these nano-scale ferric oxides and the mobility of oil droplets was further increased. The research output will help to develop a promising technology for PCB contaminated soil remediation.

Keywords: PCBs, nZVI, soil washing, remediation.

1. INTRODUCTION

As family members of chlorinated hydrocarbons, polychlorinated biphenyls (PCBs) have been widely used in hundreds of industrial and commercial applications due to their non-flammability, chemical stability, high boiling points and electrical insulating properties (Dodoo et al., 2013). Once released into the environment, PCBs are hard to be degraded, thus, are classified as persistent organic pollutants (POPs). The PCB contaminated sites not only pose an adverse impact on human health and environmental compatibility, but also lead to financial loss and reinvestment for industries and governments.

Industries have been efforts to solve individual problems and/or processes related to site remediation practices during the past years. However, most of the previous efforts were dedicated to the storage of PCB wastes at sites. According to the Federal Contaminated Sites

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Action Plan, there was a shortage of effective technologies to treat and remove PCB contaminants from soils and sediments (CSMWG, 1999), and the remediation was usually long-term and costly. This situation has hindered the efforts to effectively protect environments of this region. Therefore, it is desired that innovative technologies that can enhance efficiencies and effectiveness of remediation of PCB contaminated sites be developed.

Gomes et al. (2013) conducted a comprehensive review on remediation of PCB contaminated soils, with technologies such as incineration, landfill disposal, thermal desorption, solvent extraction and soil washing. Conventional physical/chemical remedial technologies such as incineration and landfill disposal have been frequently used, but these solutions are disruptive and unsustainable (Agarwal et al., 2007). Thermal desorption, solvent extraction and soil washing as media transfer technologies are able to transfer PCBs from the contaminated soil to a liquid or gas phase without destroying them, thus needing further dechlorination actions. Bioremediation and phytoremediation are environmentally friendly technologies that have the ability to destruct PCBs into non-toxic forms. However, complete destruction usually takes years to decades and these technologies are not suitable for heavy contamination. In recent years, more attention has been focused on technology integration. Technologies are generally applied in sequence to enhance the remediation effectiveness and efficiency. In this study, a nano zero-valent iron (nZVI) aided dechlorination, followed by soil washing approach, was tested and its corresponding remediation efficiency was investigated. The application of nZVI to the soil washing system was expected to enhance the dechlorination of PCBs, and further increase solubility of PCBs in the washing solution, leading to increased remediation efficiency, and resulting in a PCB-free washing fluid which could be easily treated further.

2. MATERIAL AND METHODS

2.1 PCB Contaminants

Transformer oil used in old transformers was collected and provided by Newfoundland Power (St. John's, Canada) for PCB contaminated soil preparation.

2.2 Physicochemical Properties of Soil

The plain soil used was fine sand obtained from a local soil company (City Sand & Gravel Ltd., St. John's, Canada). The fine sand was dried at room temperature for one week and sieved with 2 mm mesh before use.

The physicochemical properties of soil and the corresponding test methods were listed in Table 1. The soil density was calculated as 2.71 g/cm^3 according to Archimede's Principle. The bulk density was found to be 1.78 g/cm^3 by core method. The pore space was then determined to be 34.3% based on the particle density and bulk density.

Table 1. Properties of fine sands used in this study

Properties	Method	Results
Soil pH	USEPA 9045d	7.53
Moisture content	ASTM D2216-10	0.069%
Hydraulic conductivity	ASTM D2434-68	0.024cm/s

2.3 PCB Contaminated Soil Preparation

The fine sand and PCB contained transformer oil were thoroughly mixed in a stainless steel tray until reaching a homogenous phase. The tray was then covered with tin foil and stored for one month. After that, the oil in the tray was drained off until there was no fluid in the soil and the soil was ready for nZVI treatment.

2.4 Activation of the Air-Stable nZVI Particles

The nZVI particles used in this study are commercialized air-stable nano iron powders called NANOFER STAR, which were purchased from NANO IRON, s.r.o., Czech Republic. For the activation, the nZVI particles were mixed with deionized water at a ratio of 1:4. The mixture was then activated by a Branson Sonifier™ brand digital ultrasonic homogenizer for 2 minutes at 50% amplitude. The treated mixture was sealed and stored at room temperature for two days before dechlorination experiments.

2.5 Effect of nZVI Dosage on PCB Dechlorination

For each dosage, the activated nZVI slurry was transferred into a 500 mL wide neck amber glass bottle together with 200 g of PCB-contaminated soil. The solid and liquid phases were thoroughly mixed and covered with a solid-top cap. The homogenous mixture was stored at room temperature and the reaction between nZVI particles and PCBs lasted four weeks. The 5 g, 7.5 g, 10 g, 12.5 g and 15 g per kg of contaminated soil were tested, respectively, and the corresponding congener dechlorination rate was examined. Concentrations of different PCB congeners in soil were analyzed using Gas Chromatography Mass Spectrometry (GC-MS) (Agilent 7890A/5975C GCMS System). An appropriate nZVI dosage for PCB dechlorination was then selected and used for investigation of the pH effect on PCB dechlorination.

2.6 Effect of pH on PCB Dechlorination

There is a hypothesis stating that protons are essential for dechlorination and consumed during the reaction, so that an acid soil condition can enhance dechlorination (Varanasi et al., 2007). This experiment was thus designed to verify the Varansi et al. (2007) hypothesis. The initial pH level of the homogenous mixture of nZVI and the PCB contaminated soil was adjusted using sulfuric acid (Sigma-Aldrich Canada Co.) to 2 and 5, respectively. Through using selected nZVI dosage and following further experiment procedures stated in section 2.5, the effect of pH level on PCB dechlorination was examined.

2.7 Effect of nZVI on Soil Washing

Parallel experiments were conducted to investigate the effect of nZVI particles on soil washing treatment. Two types of contaminated soils were applied: 1. the original PCB contaminated soil with no nZVI treatment; and 2. the nZVI treated PCB contaminated soil. The batch scale soil washing system consists of a washing fluid reservoir, a soil column, a peristaltic pump and an effluent collection system. The Fisher Scientific™ medium-flow peristaltic pump contains variable speed drives in the range of 0.4 to 85.0 ml/min. The soil column is made of glass since the plastic materials contain phthalate esters, which will affect the PCB detection, with a

cylindrical diameter of 19 mm and 15 cm in length. The column was packed with 25 g of the nZVI treated or non-nZVI treated soil, and the outlet of the column was fitted with glass beads and glass wool to prevent soil losses during washing. The soil was washed with deionized water in a down flow mode for 1.5 hours at a steady flow rate. The washing effluent was sampled at 0, 0.5, 1 and 1.5 hours of washing, and the soil was sampled before and after the washing. All the liquid and soil samples were analyzed by GC-MS.

2.8 Sample Analysis

Duplicate water and soil samples were first extracted using hexanes (HPLC grade, Sigma-Aldrich Canada Co.) by ultrasonic extraction (EPA Method 3550B 1996). The extracts were then cleaned and concentrated through solid phase extraction (SPE). The Supelclean™ Sulfoxide SPE Tubes (Sigma-Aldrich Canada Co.) with 3 g bed weight and 6 mL volume were purchased and applied. Finally, the PCB testing was performed using GC-MS with an Agilent 7693 auto sampler. GC conditions were set up based on EPA Method 8082A (1996), with the final temperature adjusted to 300 °C to ensure no PCB congener was retained in the column. The analysis of each congener and its surrogate were carried out using Selected-Ion Monitoring (SIM) Chromatogram. The results were presented using the averages of data from duplicated analysis.

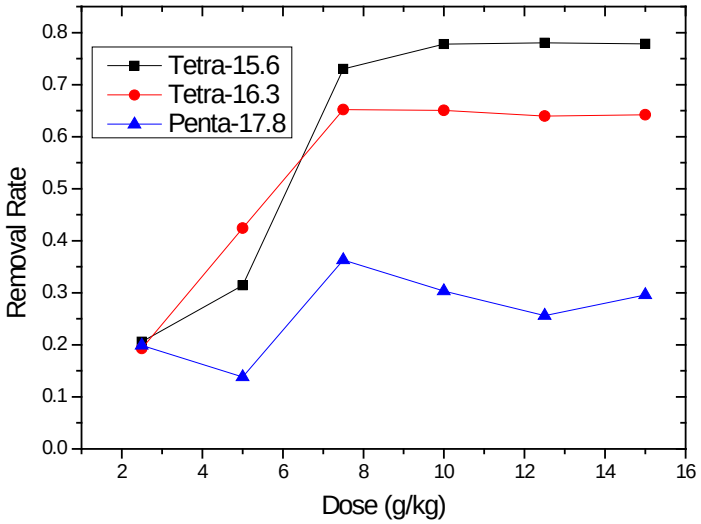
3. RESULTS AND DISCUSSION

Five PCB congeners with relatively high abundances in contaminated soil were examined, including Tetra-15.6, Tetra-16.3, Penta-17.8, Penta-18.7 and Penta-20.0. Each congener was named by the number of chlorine atoms on the biphenyl and the corresponding retention time on the MS spectra.

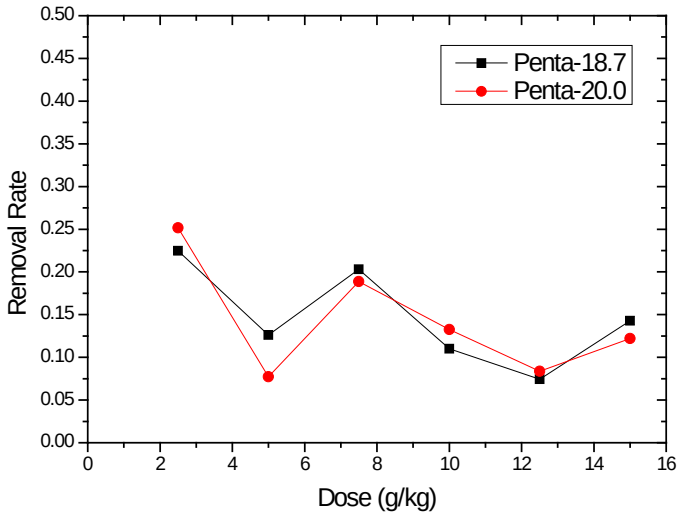
3.1 Effect of nZVI Dosage on PCB Dechlorination

The PCB with less chlorine atoms in the structure showed a much higher removal rate. As indicated in Figure 1 (a) and (b), the maximum removal rates of Tetra-15.6, Tetra-16.3 and Penta-17.8 were 78%, 65% and 36% respectively. In addition, the PCB dechlorination is also affected by the position of chlorine atoms in the structure. Once the retention time changed from 17.8 to 20.0 minutes, a decline of the PCB removal rate was observed suggesting lower dechlorination reactivity.

Figure 1 (a) shows that the trend of nZVI dosage was similar among Tetra-15.6, Tetra-16.3 and Penta-17.8. The removal rate of each congener was sharply increased with the increased nZVI dosage and became steady when the dosage was higher than 7.5 g/kg. The nZVI reached its maximum capacity of PCB dechlorination when the dosage reached 7.5 g/kg. Figure 1 (b) shows the unstable trends of Penta-18.7 and Penta-20.0 caused by the changes of the nZVI dosage. It might be due to a compromising concentration combining the deduction of reactant with the increase of product during dechlorination (Chen et al., 2014). Even though, using nZVI with a dosage of 7.5 g/kg, a better removal of PCBs was achieved. This dose was then selected for conducting the following experiments.



(a)



(b)

Figure 1. Effect of nZVI dosage on the removal of PCBs in contaminated soil

3.2 Effect of pH on PCB Dechlorination

The nZVI aided dechlorination has been extensively studied by Matheson and Tratnyek (1994), Orth and Gillham (1995), Farrell et al. (2000), as well as Deng and Hu (2002). The mechanism is shown as follows:

$$Fe^0 + \mathrm{R-Cl} + H_3O^+ \rightarrow Fe^{2+} + \mathrm{R-H} + Cl^- + H_2O$$

Generally, an acid soil with more protons might accelerate the dechlorination of PCBs. To test this hypothesis, two levels of pH were selected, with PCB dechlorination results illustrated in Figure 2. For each congener, the removal rate of PCBs at a pH level of 5 was higher than that of 2, which was not aligned with the results in the above stated publications. It might be because in this case, the proton concentration was sufficient even when pH level was 5, therefore, the pH level no longer had a dominant effect on PCB dechlorination. To the contrary, the addition of H_2SO_4 would tend to interfere with the mass transfer of PCBs from a soil to iron (Fe) surface (Varanasi et al., 2007), thus decreasing the rate of PCB dechlorination. Therefore, a pH level of 5 was selected for PCB dechlorination.

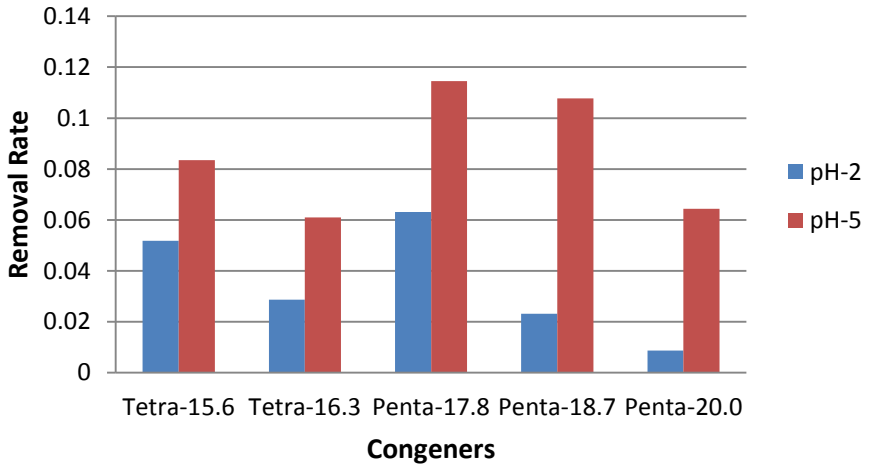


Figure 2. Effect of pH on the removal of PCBs in contaminated soil

3.3 Effect of nZVI on Soil Washing

When using the non-nZVI treated soil, the PCB concentrations in the washing effluent were extremely low (lower than 0.2 ppm), indicating that soil washing using deionized water as a solution is not directly applicable for PCB removal. Although the insolubility of PCBs makes their distribution negligible in the water phase, the transformer oil could be flushed out with limited amount of PCBs, which are consequently washed out of the column due to the high washing flow rate. As shown in Figure 4, after 1.5 hours of operation, about 16% of the original PCBs were removed by direct soil washing.

Figure 3 shows the concentration of PCBs in the washing effluent when the nZVI treated soil was washed for 1.5 hours. A red color was observed in the treated soil, implying the formation of

ferric hydroxides or ferric oxides. It indicated that the nZVI particles were transferred to their oxidative forms after the dechlorination reaction. During soil washing, the PCB concentration for each congener decreased such as for Tetra-16.3, with the relative concentration at the 0.5 hour of operation being 0.057, and decreasing to 0.004 at 1.5 hour. Figure 4 shows the PCB concentrations in soil after washing. The removal rate of PCBs by soil washing was 63.0%, 62.8%, 61.7%, 62.0%, and 59.4%, respectively, in the five congeners in nZVI treated soil in comparison with that of the untreated one. It was illustrated that the presence of nZVI greatly enhanced the soil washing efficiency, however, the presence of nano-scale ferric oxides in the system also played key role in PCB removal. The contaminated soil trapped a certain amount of transformer oil, and the oil droplets were blocked by the pore throat of soil due to the high interfacial tension of oil and soil (Roustaei et al., 2013). Additionally, with the presence of nano-scale ferric oxides, the interfacial tension would be reduced and the mobility of oil droplets would increase (Hendraningrat and Torsæter, 2014). As a result, more oil droplets were desorbed from soil, resulting in the increased effectiveness of soil washing.

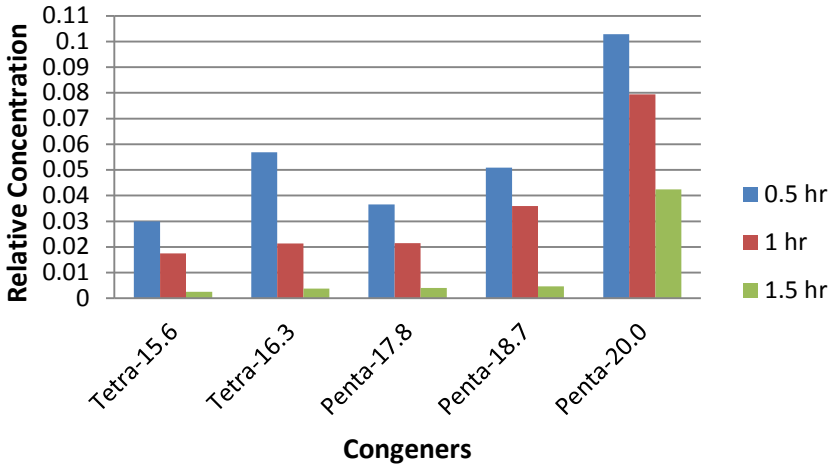


Figure 3. PCB concentration in the nZVI treated soil washing effluent

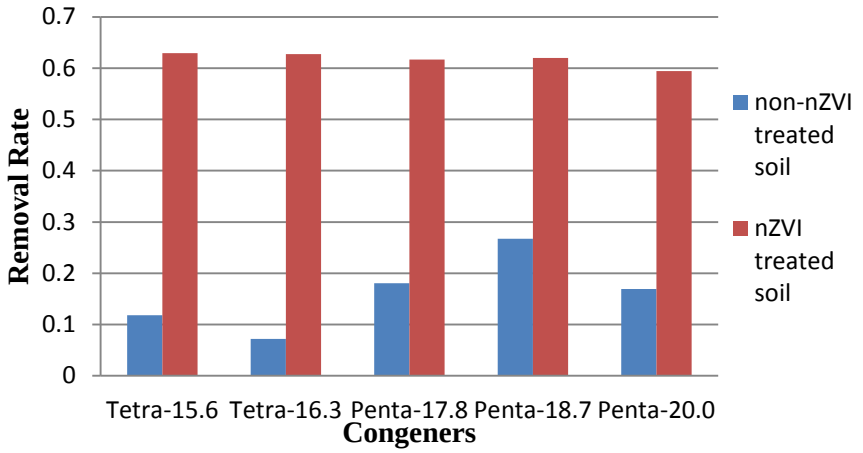


Figure 4. Washing efficiencies of non-nZVI treated soil and nZVI treated soil

4. CONCLUSIONS

In this study, the nZVI aided dechlorination integrated with a soil washing treatment was tested for PCB removal from soils. Results based on five different PCB congeners indicated that an integrated method can greatly enhance the treatment effectiveness. In terms of nZVI dechlorination, the effect of the nZVI dose and initial pH level was evaluated. A higher nZVI dosage led to a higher dechlorination of PCB in the congeners when the dosage was lower than 7.5 g/kg soil. The highest dechlorination rate of about 80% was found in Tetra-15.6. Congeners with lower chlorine atoms (Tetra-15.6 and Tetra-16.3) had higher dechlorination rates in comparison with congeners containing more chlorine atoms (Penta-17.8, Penta-18.7, and Penta-20.0), which proved that lower chlorinated biphenyls are easier to be dechlorinated. The appropriate nZVI dosage for PCB declorination was proven to be 7.5 g/kg soil, with the removal rates of Tetra-15.6 and Tetra-16.3 being 75% and 65% respectively. Results also indicated that a pH level of 5 was better for PCB dechlorination in comparison with a pH level of 2. The presence of ferric oxides formed after dechlorination were capable of enhancing PCB removal in soil washing due to the interfacial tension between the oil droplets and soil which were reduced by the nano-scale ferric oxides and further increasing the mobility of oil droplets. The washing operation could remove as much as 63% of the PCB congener from the nZVI treated soil. The research output would help to develop a promising technology for PCB contaminated soil remediation.

5. ACKNOWLEDGEMENT

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SUSTAINABLE PLANNING AND AVOIDING PITFALLS

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ABSTRACT

When making Brownfields "green" or sustainable, three key concepts need to be addressed - social equity, economics, and ecological/environmental health. Through a collaborative team, a wide variety of disciplines and ideas can be drawn from instead of relying solely on city planners to make decisions in a vacuum. Successfully greening brownfields is a team effort and this presentation provides helpful tips to this multifaceted process.

The process should identify the many assets, supporters, and plan elements for sustainable redevelopment and can be a bit larger than first imagined. There are stakeholders and resources, which are often not considered, that are available to a community or redevelopment project. These stakeholders and resources could include a wide variety of people, from residents to businesses to government entities. Each offers a different sustainable attribute to the community and a new perspective if brought together in a productive way. Their interaction, expertise, resources, and ideas come from a perceived need or asset in the community. By understanding these aspects, a redevelopment can actually reduce costs and increase sustainable attributes, such as LEED certification. In this presentation, we will provide a brief guide to sustainable, strategic planning and implementation to help a community understand where to begin, some of the many potential resources available, and some important opportunities to be successful.

Also, it is important to identify the pitfalls of a redevelopment project to avoid costly mistakes and issues. A fashionable bioretention pond or a wetland for storm water management may not be appropriate for your site. Identifying misalignments in your development can ensure maintenance of a budget and success. This presentation will provide examples of misalignments and an overview of sustainable misalignments, and environmental requirements that should be reconciled with your sustainable vision to ensure you remain within budget and have a successful redevelopment project.

Keywords: Sustainable; Sustainability; Planning; LEED; Community; Brownfield; Greenfield; Redevelopment; Remedy; Strategic Planning; Pitfalls

1. INTRODUCTION

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Sustainable planning can be a daunting task and can leave a city unsatisfied with the results. Granters and funders may be unhappy that funds have been provided, but the redevelopment did not return the expected investment they hoped. Many of these concerns can be avoided by proper sustainable planning and development of the appropriate project vision. Planning has also become a hurry to incorporate the newest sustainable trend, but sometimes this piece can be more detrimental than helpful to your community. While sustainability is a good thing overall, sustainability just for the sake of being part of the new revolution may not. Sustainable concepts may be complex in nature and are not one size fits all. In this discussion, we will look at good and bad planning and implementation of sustainable aspects.

Let's first understand the basics of sustainability; Webster's Dictionary defines "sustainability" as "… of, relating to, or being a method of harvesting or using a resource so that the resource is not depleted or permanently damaged…b: of or relating to a lifestyle involving the use of sustainable methods."(Merriam-Websters, 2014). Here we see that sustainability is a concept of keeping balance with the world around you through not taking more than the world can support. When planning, a community needs to evaluate the resources that it has and how best to protect those resources as it moves into planning. An example is a Great Lakes city which needs to address redevelopment along the lakefront. This resource can be used for recreation, drinking water, and power so it would be an important resource to protect. However, the rust-belt city's past saw industrial development along the river's mouth and the shoreline which has caused multiple complications to redevelopment. So what is the best and highest use your planning team can expect? How do you redevelop or realign areas of concern for the best vision of the community? Here a planning team needs to understand how the resource is currently impacted, what impacts can the redevelopment address and/or eliminate, and what are the community's needs that could best be addressed by this redevelopment? The planning team needs to begin by evaluating and considering all aspects in their redevelopment. Beginning your planning with sustainability in mind can reduce costs instead of adding costs amidst change during a project. Additionally, the planning team should determine the stakeholders that are affected by the project and include their input during the planning stage. This can offer new ideas and allow for flexibility in a project as it unfolds.

An example is the redevelopment occurring at the Flats East project in Cleveland; an old industrial area along the mouth of the Cuyahoga River leading to Lake Erie turned into an entertainment area which was trending down in the economic recession. A developer saw a much brighter and sustainable use for this property through redevelopment into a mixed-use area. The brownfield would be remediated, new office and mix-use facilities would be created, and green infrastructure would address river quality, as well as green bulk heading for recreational fish species. This higher use would increase the economic value of the area, return residents to the city instead of the suburbs, provide for clean-up of a brownfield property, and include green structures to improve ecological habitats and river quality. However, the housing market crash and 2008 recession hurt the redevelopment. The developer had early on identified multiple stakeholders including local, state, and federal agencies and commercial businesses to support the redevelopment. With a focus on green infrastructure and sustainable attributes, there was more interest in the project from these additional stakeholders. So although banks were not lending and residential development was not moving, the additional stakeholders allowed the project to be refocused during this time to business purposes with public money for green cleanup and redevelopment. The project changed focus and continued to move forward until the market constraints began to change to support mixed-use redevelopment. These upfront

sustainable planning techniques allowed for flexibility and support to the project and garnished interest based upon sustainable attributes of green building and LEED certification (lowering operational costs of the buildings).

Sustainability also provides for the balance in lifestyles to ensure that a way of life does not upset the balance of those around it. In effect, it is the consideration of how the action or lifestyle affects everything around and does not place more burden on one aspect more than another. Environmental Justice (EJ) is a sustainable concept since it addresses the use of a disadvantaged community to house bad environmental industry practices due to lack of political support. The EJ concept focuses on not harming one population to benefit another, such as a community locating a chemical company, for a tax benefit, in the lower income area to benefit the larger populace. This example shows the inequity to those who do not have higher paying jobs in the community and therefore are not thought of as important to the community. However, these people can support cultural diversity, service jobs, and new ideas, and they can benefit from volunteer and redevelopment activities in the community and bring much needed commitment to community projects. Meanwhile higher paid professionals support tax bases and business development, but they are not usually as grounded in the community.

Finally, let's focus on the global perspective, and the three key elements that need to be combined for true sustainability – Environment, Economic, and Equity (social). However, the balance of this important concept is difficult to obtain. Let's look at this balance in terms of human health – if a person has an inner ear infection it will compromise their balance and other parts of their life, or a person may run a fever, feel nauseated, or have headaches, all of which are related to an ear infection. Have you ever gone to the doctor with symptoms and received multiple options that could cause your discomfort? Each symptom is investigated through medical testing to narrow down the cause. This is similar in a community, as a community needs to better understand itself to properly balance the sustainable aspects of a development or redevelopment project. Just as the inner ear infection has many symptoms common with other issues, a brownfield area or greying of a community show diversity issues that can lead to several different causes. By selecting the wrong cause or only one of multiple causes, a community's balance may not be realized.

2. PROCEDURE

So how would a community begin this process? Most communities have completed Master Planning for their city or town. This process is usually completed by the city planner, zoning director, and/or building commissioner. If the city is large enough, it may have a community development and economic development director that are also key to this process. The process may have included public comment or notice and have been part of a council meeting. The planning may be directed by the mayor or local government. However, in most cases, it is driven by a small number of participants identifying community issues with a focus based upon their needs. They may not have evaluated the planning on a larger scale or included outside stakeholders that could make the planning more flexible, driven, and sustainable because they do not have all the expertise and resources to do so. So let's look, as a process, to expand the ability of communities to find the expertise and resources which may drive a more sustainable strategic planning process.

2.1 Defining and Scoping Your Project

First, a community must identify the scope of their project planning to ensure it is achievable and their needs are within the project scope. Reviewing current or previous Master Plans for the community is a good place to start to identify how the community has determined their assets and needs now, and in the past. The next step is to compile a list of current community issues and needed changes to address the items of concern for the community. Also examine all facets of stakeholders in your community as many governments only look to the voters and community activists and not to local businesses, neighboring communities, or regional partners.

This initial data collection phase of project design should include understanding your community assets, needs, and debit, as well as all the possible stakeholders in the process. Remember your stakeholder list may initially be very large and include anyone who could be impacted by change in your community. The size or acreage of the project is also important so you don't overextend your resources, so begin by determining an initial area your team wants to focus on. Remember this process is fluid and the size could change as you move forward. For example, a community can look at redevelopment designs for the community as a whole, a portion of a neighborhood, a small bounded area of the community, or even a building location that has been identified as a concern for the community. Also during the planning process, the community would want to consider the purpose or purposes behind your planning, such as to spur redevelopment of an area or address an eyesore within the redevelopment area. In Atlanta, an old rail line was identified as an eyesore and safety concern, however, there was also an underlying purpose to connect neighborhoods and provide green space. These purposes combined for the belt project that is redefining Atlanta's outer ring.

To complete your first step in the process, the following items need to be defined- a mission statement, project and team boundaries, stakeholders, and assets and concerns that drive project selection. Keep these items general and open to change as you will refine these items during this iterative process of project planning and implementation. Flexibility is the key to keep moving forward with limited delays.

How would this look in practice? For example, a community has multiple closed gasoline stations that provide eyesores to the city and the community is looking to develop a master plan to address this issue. The local council has presented the opening of their planning process to the community through a local newspaper announcement and held a council meeting. During the council meeting, several citizens noted that they would like community gardens and urban agriculture opportunities, which have been touted in a nearby city. Also, the police and fire department representatives have identified these locations as trouble spots for crime. Additionally, during a zoning meeting, a local business notes that they are looking to relocate as they need to expand and address employees' needs. Employees have complained that they need to travel outside the city for recreational and lunch time opportunities as these facilities are not within the area of the business. The community has now heard some of the concerns and needs of their stakeholders. This is only a small sampling of the information gathering that occurs during the planning process. At this stage the planning department should determine how they want to continue. The first question should be does the team want the focus of this project to be – developing a community-wide Master Plan, focusing on only the gas station issue, or focusing on the loss of a business? These issues could be the same, intertwined, or completely different

projects. So how does a community develop a scope for the different efforts identified in the initial data collection step?

2.2 Project Data Collection and Data Analysis

Once the scope of the planning is determined, the information gathering can begin through two key initial actions. One step is for the planning group to identify all assets and debits of the project. This means to make a list of what is good and should be kept as part of the final plan, and what is missing or needs to be changed. A simple spreadsheet could be used, but remember this is an ITERATIVE process and this list could change as the planning moves forward. The second step is to determine what stakeholders should be included as part of the planning process. As stated above, this is not a short list and should always consider everyone during the brainstorm period. Examples could be residents, citizen groups, businesses, business groups, government departments, neighboring community members and quasi-government/non-profits, regional entities (park service, county governments, etc), interested developers or businesses that may come into your community, community services (churches, schools, healthcare facilities), and any other users or visitors to your community. Yes, this is a large list, but in the beginning you want as much information as possible to develop ideas. This may also identify resources and leadership to spur the different phases of redevelopment as the project moves forward.

The next step is to gather the data for project planning. This could entail community meetings (charettes), surveys, or other data collection methods. The group should initially include all stakeholders and consist of a focused questionnaire. The idea is to develop a vision for the area that is flexible and feasible, but don't throw out any information at this early stage. Identify what is important to each stakeholder, what changes they would like to see, and what they feel is important not to change in the planning. This may include a historic aspect of the area the residents feel define the cultural heritage or history of the community; a medical facility they need to support the residents; a retail district to support residential and commercial customers; or greenspace to beautify the community. As you move forward in your information gathering, a core group for your planning team may be identified. These are people that are truly committed to the cause of redevelopment and can be valuable support to making the project happen. Government participants can be the facilitators to keep this group together and assist in solving conflicts. These may be the leadership and funders that could propel the project (s) to success in your community. The group will go through the brainstorming and norming process to develop several options for redeveloping the area. As the team completes the planning process, they need to identify key items for the plan – Funding, Sustainable Aspects, and Support Resources. Part of the initial stakeholder list may hold key people that could be contacted to help with these items.

As the main elements are derived from the planning group, leadership on various areas may become evident. While government officials may provide a good start and facilitate the planning, they often do not have the time or resources to fully accomplish the projects that may come from the planning process. The stakeholder group, as it develops, may identify community leaders or activists that can lead the charge to getting urban gardens developed, a playground built, or new business opportunities identified and negotiated for a community. Valuable expertise and resources can come from regional government involvement, foundations and philanthropic organizations, and industry/business partners. Community development does not

work if the only partners are public entities, as a public-private partnership provides the stability and resources to boost a project's success.

An example is when a community has identified a need to provide better food for its inner city children. During stakeholder meetings, a local industry identifies they can provide kitchen products and training to help the school and community centers that provide meals. A community volunteer group suggests development of an urban garden area to grow fresh foods, and volunteers can work with children and community members to educate them on agricultural methods. A school superintendent welcomes the agricultural programs from both industry and local gardening groups, and pledges school resources for a new garden, time during school for children to participate, and transportation for offsite opportunities. One need is met without the need for government funds or direction, but came out of the government-led stakeholder meetings.

As these opportunities are realized, another issue may arise for a community. As they identify the number of projects that the community would like to pursue, they may find it is daunting to pursue them all at once. A priority system needs to be developed to define which projects should be the highest priority to complete first and focus resources towards. Here stakeholders may not agree and this could be a frustrating task; however, when completing this prioritization, it is important to define the projects that could impact other projects. Let's say your community is looking at two projects – one is an old gasoline station on the main road into town and another is an old industrial area near a new residential development that has already enjoyed success. The two projects may both have significant support, but your planning committee can only handle one. The key is to look at which one will provide the most "bang for the buck". Investigate the underlying aspects of both projects and determine which could spur additional development or be more protective of the community's future. Will the redevelopment of the gasoline station provide a resurgence of business opportunities to increase employment in the community and provide a better image to your town? Does the industrial site pose a health risk for new residents moving into your community which could hurt the community's growth potential? Look at the return on investment of both projects for the cost you would incur, and then begin to prioritize them to the betterment of your community.

It should be noted here that costs are not the only consideration for your return on investment. A community needs to look at marketing towards additional business or residential development, community relations, governmental relations, community needs, and other areas that impact daily quality of life for a community. The community may also find that project priorities can change through time. A project that was a high priority may need to be realigned if a new issue or change in resources occurs. An example is a community has a business that wants to come into the community through a redevelopment project. This may be a high priority for the community until the business pulls out of the deal and moves operations elsewhere. So again, it is key to be flexible and ensure there are multiple options on the table so you can move on if conditions change. An iterative and flexible process needs to be the focus of sustainable planning.

2.3 Decision Making

Sustainable decisions can provide higher return on investment and greater interest in today's market. As you prioritize your main projects and develop funding and resources to complete the projects, the planning team should also look at the sustainability of the development or

redevelopment of the project. The triple bottom line aspects are important to ensure your community does not have to relook at this area again in 5, 10, or 20 years. Today's triple bottom line strategy includes the development strategy aspects that minimize operation and maintenance costs, human costs (health care, loss production time), and garnish social acceptance to support consumer loyalty. Greening your community can offer a more aesthetically pleasing venue for business and residents, and green buildings provide healthier environments with lower operational and maintenance costs over time. The U.S. Green Building Council (Leed, 2012-2014)[3] has developed the Leadership in Energy and Environmental Design (LEED) rating system to help identify building or neighborhood green standards. However, a good understanding of the development project and its limitations is very important. When making decisions on developments, green components can accentuate the value of the project and help to keep the end use viable into the future. It has been identified by multiple sources that green buildings and neighbors are more marketable in today's economy. They also include elements to allow flexibility to grow and change with the building use and community needs. Green aspects include alternative energy and resource options that could support a community's infrastructure. A green redevelopment design can reduce stormwater conveyance and demand on sewer systems, reuse greywater to reduce the demand on potable water supplies, and green energy alternatives, like solar, can even supplement the utility grids of a community. So a community may want to look at building regulations to require new developers to consider green options in their construction designs. Also, communities can support green building options using tax incentives and fee reductions for green developments.

However, poor choices in decisions can also impact your community development strategies. Some projects do not support certain green alternatives, and implementing green aspects for the sake of "being green" can leave a community with more problems than solutions. In planning, the quickest process to be green may not be your friend. As communities and developers look at including possible green alternatives, there needs to be a review of the property and surrounding area to see if these are viable options to be employed. Green infrastructure to increase infiltration may not be appropriate for a brownfield, or a green roof may not be viable for the type of building. Green structures need planning and design time and should be considered at the very beginning of the design process. Early integration of these elements can reduce costs, ensure success, and modify other systems for additional savings and design success. The later green alternatives are considered the more the realization of increased costs may be incurred, as to modify designs for these elements would incur higher costs.

2.4 Real Life Examples – Good and Bad

To aid the reader, examples always help to accentuate the positive and negative facets of redevelopment projects. Here we will highlight some of the good and bad attributes of project planning and design in community projects. On a positive note, several projects have turned a negative aspect of their community into their advantage. In Cleveland, the city and port authority have begun reuse of confined disposal facility (CDF) sediments (U.S. Army Corp of Engineering's river dredging) for reuse on upland projects to help provide fill materials on contaminated sites. If you are interested in a good case on reuse of CDF areas, the Dike 14 project highlights how a CDF can become a natural area for recreation. Also, Cleveland is continuing reuse of sediments at another dike area to provide additional fill materials for

redevelopment projects throughout the area. This reuse is saving money, reusing waste materials in a beneficial way, and freeing dikes for additional use by the U.S. Army Corp of Engineers.

In Columbus, the Whittier Peninsula project changed a brownfield into a green standard park setting. The old industrial property, which had contaminated environmental media, was redeveloped into additional commercial properties and a metropolitan park with a LEED rated park education center. The education building includes a green roof, sustainable green infrastructures for storm water management, and other sustainable attributes to educate the visitors on nature and sustainability. The strategy began by evaluating the cost effective options for cleanup of the property and aligning those strategies with reuse options. Areas where greater contamination concentrations were located were reserved for commercial/industrial use, and areas that could be remediated to a higher use in a cost effective manner were identified for the recreational facilities. Drainage and grading were designed to reduce impacts to the river adjacent to the property.

Finally, an easy and cost effective example that can be added to almost every demolition project is deconstruction. In Middletown and Painesville, Ohio, two redevelopment teams of public and private stakeholders used deconstruction techniques to save money and reduce wastes entering into local landfills. These projects recycled and reused as much of two old hospitals as possible to save hundreds of thousands of dollars while diverting thousands of tons of solid waste from local landfills. The process included recycling wood, metal, and concrete and reusing cabinets, beds, and equipment offered to local businesses and residents. These success stories are only a small sample of what sustainable strategic planning can do for a community.

For redevelopment strategies, the Flats project mentioned earlier in this article is both a positive and negative example of sustainable planning. As noted earlier, strategic planning and flexibility built into the project provided protection from changing economic factors. The sustainable aspects of the project, including LEED certification, increased the value of the property and its marketing ability. However, during the design, trouble arose when project teams did not interact. As the development team endeavored to make the project design meet LEED standards with stormwater retention features, they did not account for the brownfield issues and remedy design occurring in a different phase of the project. As recommended, the development team looked for all sustainable aspects to include at an early stage; however, they did not evaluate the property and its limitation when designing. The team included infiltration attributes of green design that impacted remediation and water quality at the property. The project's environmental remediation included cell designs for leaving contaminated materials in place in lieu of costly removal and disposal. However, this design required ensuring water infiltration would not impact migration to ground and surface waters and this was in direct conflict with development plans. During a regulatory review, this issue was identified and revision of the development was implemented. However, this shows that project teams need to communicate and all designers need to understand the property or project area and its potential limitations in design.

Finally, poor examples usually occur when communication is lacking, understanding of the full project properties is lacking, and when planning is affected by timing. A developer in Ohio wanted to move quickly to install a retail area, but was required to use green infrastructure. The developer used permeable pavers as part of his parking area, but did not understand the maintenance requirements or the underlying material at the property. Within a year, regulatory inspectors found issues of run off from the parking lot due to underlying petroleum contamination at the site and flooding was occurring due to lack of maintenance of the pavers.

This cost the owner money to replace the parking area with standard concrete and revise the stormwater attributes of the property to meet surface water regulations.

As with the Flats example, another retail project was redeveloping a brownfield area in Cleveland. The previous use was part of a large steel mill and the property contained a significant amount of slag fill. In development, a regulatory agency required use of green infrastructure features, however, the agency did not understand the underlying conditions of the property. The developer appealed to the regulator overseeing the remediation of the site to help educate the stormwater regulatory body. In this case, new alternatives were explored to meet the stormwater green requirements since implementation of bioretention ponds and permeable surfaces would cause increased impacts to ground and surface waters underlying and adjacent to the contaminated property. Additionally, the developer could have looked at reuse of stormwater on the property to see if surface runoff could be captured to reduce additional use of potable municipal water and reduce operational costs.

3. CONCLUSIONS

Planning, communication, and a good understanding of your project from the design stage are all important concepts in ensure the sustainable development or redevelopment projects are a success. Beginning with understanding your community, its assets and needs, its full range of potential stakeholders, and the goals of your planning are also important first steps. This planning process allows a community to take full advantage of its resources, both human and capital, and explore many alternatives and ideas for the best possible options as you move forward in your strategic planning process. Also, as members of a stakeholder group communicate new funding sources, other resource options, operation-sharing opportunities, business opportunities, and community support can all be identified to make the project move towards a successful endpoint. Flexibility and sustainable elements provide for continued use of a property so you do not have to continually revise your community master plan.

Additionally, understand the triple bottom line economic aspects to provide more value to the project. Green or sustainable traits can benefit marketing strategies of properties by highlighting lower operational and maintenance costs, better environmental quality for users and visitors, and healthy living features, such as pedestrian/bike friendly transportation corridors, urban gardens, recreational facilities, and availability of fresh produce. Adding these traits as design requirements can help your community be more sustainable over time. Also, by identifying areas that are in need of these items, which could be a priority for your community and help revitalize community areas, - could spur more interest by developers and businesses.

Finally, our examples show the benefit of a good understanding of the project and its limitations. Sustainable features are good when used correctly, and cost less to include in your design if you plan for them early. Finding that you could have saved energy costs by redesigning your building ventilation system at the beginning of the project does not help you at the end. Nor does identifying the green infrastructure element you included in the design has caused more environmental harm than good. So every planning plan team needs to fully understand their project and communicate effectively. Sustainability is all about balance: in your team; in your priorities; in your project; and your interaction with the environment!

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