Characterization, Identification and Seasonal Evaluation of Microbes in Mercury Contaminated Soils

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ABSTRACT

Mercury (Hg) contamination within the Oak Ridge Reservation has been an ongoing research. A comprehensive understanding of the potentials of predominant microorganisms in selected cleanup strategy is highly paramount. The use of sorbents has been proposed as a cleanup strategy; however, biological factors need to be evaluated as regards to the strategy. The goal of this study was to use microbes isolated from various contaminated sites and determine their effectiveness on sorbents to reduce total Hg (HgT). We isolated DNA from contaminated soils (SB 5-8, SB 14-8) and control soil (Hinds Creek) with little or no Hg contamination. Pure cultures were obtained from soil samples using broad-spectrum nutrient broth, and natural medium formulated using extracts from the different soil samples. Selected colonies and total DNA from the soils were used for 16S rDNA sequencing. Some Hg reducers (*Acinetobacter rhizosphaerae* and *Serratia marcescens*) were isolated. *S. marcescens* was chosen due to its role in bioremediating and transforming Hg. Study of hg leachability in the contaminated soils showed that the amount of leachable Hg varied in magnitude among the three soils possibly due to seasonal changes. Sorbent was added to determine control in leachability after the soils have been incubated for 14 days in diH_2O. *S. marcescens* was inoculated into the soils along with sorbents and incubated for 48 hrs at 25^oC. Total Hg (HgT) concentrations were determined using cold vapor atomic fluorescence in accordance with EPA method 1631. Variable amount of methylators was found in each soil type. Organisms that grew on the nutrient agar grew poorly on the natural medium with variable colonies. However, *S. marcescens* grew on both media. *S. marcescens* showed high reduction of Hg^{2+} compared to the sorbent; however, when inoculated in the soil, the sorbent was found to absorb more HgT in solution.

KEYWORDS

Total Mercury, Sorbents, Bioremediation, Leachability, Methylators, Aerobic microbes

INTRODUCTION AND BACKGROUND

The Y-12 National Security Complex around the Oak Ridge Reservation has been identified by the US Environmental Protection Agency (EPA) to have heavily contaminated the upper and lower East Fork Poplar Creek (EFPC) ecosystem with Hg through transportation from the source areas via atmospheric deposition, sediment transport, surface water runoff, and groundwater transport contaminating the upper and lower EFPC ecosystem [He et al 2010].

Part of the current strategy to remediate of Hg from the EFPC is the use of sorbents to immobilize Hg from the EFPC. Tests conducted on various sorbents showed varying level of Hg affinity. Sorbents such as Thiol-SAMMS and Organoclay have been identified to have great ability to immobilize organic Hg, Hg^{+} and Hg^{+2} [Chen et al 1999; Wang and Abraham 2009] among others.

Hg is one of the most dangerous metals that freely exist in the environment. Hg pollution occurs through the release of fossil fuel globally with 90% of total Hg (HgT) released to the atmosphere due to steady combustion [Pirrone et al 2010]. Hg can be found in soils, aquatic environments, and sewage. However, in soils Hg is more persistent compared to the aquatic ecosystems and sewage [Xu et al 2015]. For the most part, Hg enters the food chain through soils or water thereby increasing health risks [Cui et al 2011]. Hg originating from both the aquatic and terrestrial environments can seriously endanger the food chain and degrade the ecosystem thereby threatening human health. This in return increases the need to transform the Hg in the environment into its most stable and less toxic form.

Hg exists in three (organic, inorganic and elemental Hg) forms. Organic Hg results from Hg combining with carbon and other elements. The most common and toxic form of organic Hg is methylmercury (MeHg) [Reinfeldera et al 1998]. Bacteria methylate Hg by the excretion of methylcobalamin [Robinson and Tuovinen 1984]. The production of MeHg is predominant among microorganisms especially anaerobes [Hsu-Kim et al 2013; Gilmour et al 2013; Podar et al 2015]. Moreover, obligate anaerobes are mostly associated with Hg methylation and they include diverse groups such as sulfate reducers, iron reducers, methanogens, syntrophs, acetogens [Gilmour et al 2013; Lee et al 2016; Jeremiason et al 2006; Marvin-DiPasquale et al 2014; Gilmour et al 2011; Yu et al 2013; Yu et al 2012]. Inorganic Hg is more soluble and more reactive than the other forms of Hg. Inorganic compounds exist as mercuric sulfide (HgS), mercuric oxide (HgO) and mercuric chloride ($HgCl_2$). Several bacteria strains possess reductase genes that are encoded by *mer* operon that aids in reducing Hg^{2+} to Hg^{0} [Barkay et al 2003; Lin

et al 2012; Tottey et al 2007].

On the other hand, organisms need some heavy metals (nickel, iron, zinc and copper) in small amounts for their metabolic activity; other metals (Hg, silver and cadmium) have no role in microbial metabolism. However, at low concentration they can be metabolized [Ehrlich 1997]. Factors such as pH, metal concentration, speciation and organic matter can influence on microorganisms [Nwuche and Ugoji 2008; Sterritt and Lester 1980]. Studies have also shown that the presence of these factors in Hg contaminated environments can create a huge impact on the ecosystems [Sobolev and Begonia 2008].

In this study, we characterized the soil properties to better understand its biotic and abiotic characteristics. To understand the long-term stability of total Mercury (HgT) in the soils during various seasons; a study was performed to determine the leachability of HgT through wet and dry analyses. We also developed an easy and bio-friendly approach to isolate aerobic Hg resistant microorganisms that can transform Hg either via metabolically dependent or independent processes; or as a potential target for bioremediation. We were also able to develop a more quantitative and qualitative method to isolate genomic DNA (gDNA) from the soil samples.

EXPERIMENTAL DESIGN

Soil collection

Bank soils contaminated with Hg from EFPC was collected from two sampling sites with soil profiles based on soil color. For soil profile, the samples were collected from two distinct soil layers (Fig. 1), the top lighted-colored soil labeled SB 14-8 downstream of the creek and the bottom dark colored soil upstream of the creek labeled SB 5-8. Soil samples from the uncontaminated Hinds Creek (45 km away from EFPC) with similar general chemistry, hydrology and underlying geology as the contaminated sites were included as control. Samples were collected in summer 2015, fall 2016 and winter 2017. The samples were transported on dry ice to Alabama State University within 24 hours of sampling and kept at -20^{O}C until analysis.

Determination of the physical characteristics of soils and stream water

Hg in the soils was analyzed according to EPA method 1631 using a Brooks Rand MERX Cold Vapor Atomic Fluorescence Spectrophotometer (CVAFS) system. MeHg was analyzed using modified EPA method 1630 on a Brooks Rand MERX MeHg instrument coupled with a Perkin Elmer Elan-DRC ICP-MS. The pH was determined with a glass electrode according to standard methods. Total carbon (C), sulfurs, and nitrogen (N) were determined by the dry combustion method using a Leco 2000 CNS analyzer equipped with thermal conductivity detector. Major cations were analyzed in accordance to standard procedure using sodium peroxide fusion method coupled with Varian 735ES ICP-OES detection. A multi-parameter sonde (TROLL 9500 series) was used to record pH, specific conductivity, oxidation-reduction potential and temperature. Particle size distribution (PSD), Atterberg limits and bulk density of samples were determined in accordance with the American Society of Testing and Materials (ASTM) standard test methods D422, F1632, and D2937 respectively.

Leachability of Hg under wet and dry conditions

To examine the amount of leachable Hg from the soil samples (SB 5-8, SB 14-8 and Hinds Creek) under wet or dry conditions, soils were leached with artificial creek water (ACW). The ACW is composed of deionized water with 1.93 mg/L $Ca(NO3)_2$ to simulate the ionic

strength and the major anion and cation composition present in EFPC. A preliminary experiment was conducted in which the concentration of leachable Hg was measured from naturally wet soils and soils dried at 60^{oC} for 3 days. The wet conditions were conducted on the soil samples, while the dry conditions were conducted on the SB 5-8 and Hinds Creek soils. The leachability of dry HgS mixed with sand was also tested. The ACW was added to the samples and shaken for 24 hours, the samples subsequently filtered (GFF filter) and mercury content in the aqueous phase determined. Soils samples were either dried at 30^{oC} for 5 days or left under wet conditions for 5 days. ACW was added to all samples on day 6 and shaken for 24 hours and then filtered on day 7. This process was repeated until the amount of Hg leached from the samples reached a steady state.

Isolation of microorganisms

The soils were cultured in a non-organism specific nutrient broth used for pure culture isolation. Approximately, 1 g of the soil was transferred to 99 mL of nutrient broth and incubated overnight, ≤18 hrs at $25^{O}C$ on a rotary shaker and about 1 mL of the overnight culture grown to 10^{-6} was plated on both selective and non-selective solid media (Remel INC, San Diego, CA and WARD's science, Rochester, NY) respectively, and incubated at $25^{o\ C}$ for 24 hrs. Pure bacteria colony was repeatedly transferred to fresh solid media until single colonies were isolated. The isolates were suspended in sterile saline solution until further testing. Morphological studies of the isolates were performed using a Nikon compound high power microscope (1000 x), followed by Gram staining. Chemical characterizations of the isolates were determined based on the Bergey's Manual of Determinative Bacteriology.

Bacterial Enumeration

The soil extracts formulated using 500 g of soil was added to 1 L of 50 mM NaOH solution and incubated at room temperature overnight. The supernatant was separated through decantation and centrifuged for 1.5 hours at 14,000 rpm. Approximately 7.5 g/L of agar was added in 250 mL of soil extract and sterilized by autoclaving. The culture was serially diluted to 10^{-6} in 0.85% (w/v) NaCl. The 0.1 mL dilution was plated on the formulated agar and incubated at 25^{oC} for 72 hrs. Colony formation was recorded after 24, 48 and 72 hrs.

DNA Extraction

Soils from contaminated and reference sites were used for total community genomic DNA (gDNA) extraction at ~1 g of each sample (wet). The soils were homogenized prior to subsequent studies. A survey of the total DNA in the soil was performed using gDNA extracted from soil using PowerSoilTM DNA Isolation Kit (QIAGEN, Carlsbad, CA), with the addition of a few optimizations, and quantification using N200 spectrophotometer for soil (MP Biomedicals) in

accordance to the manufacturer's instructions. The gDNA was concentrated and further purified by precipitation with ethanol. The quality and concentration of the generated DNA was evaluated using NanoDrop N200 spectrophotometer (Thermoscientific, Wilmington, DE). The isolated DNA was stored at -20^{oC} for further analysis using 125-nucleotide paired-end multiplex sequencing on the Illumina platform.

Mercury Biosorption using Sorbents and Microorganism

All glass wares were subjected to 10% HCl and 25% HNO_3 overnight washing before sterilization to purge any traces of Hg. Microorganisms were grown between OD_{600} 0.4 to 0.6 before inoculation into the culture. About 50 ng/L of elemental Hg was used to analyze the amount of Hg that can be absorbed by either the microorganisms, the sorbents (Thiol-SAMMS™ and Organoclay^{PM-199}) (Oak Ridge National Lab. Oak Ridge, TN) (Table 1). The culture was incubated at 25 ^{O}C for 48 hours on a rotary incubator, spun down at 4,000 rpm for 15 mins, collecting the supernatant and preserving with 0.1% metal grade HCl until further analysis.

RESULTS AND DISCUSSION

Determination of chemical and physical characteristics of soil and stream water

In our initial experiments, we determined the chemical and physical characteristics of the soils. The concentration of HgT in soils was slightly greater in SB 5-8 sample (14.268 ug/gdw) compared to SB 14-8 (13.468 ug/gdw) (Fig. 6). However, the MeHgT in the SB 14-8 was twice the amount in SB 5-8. The pH of the soils and stream water was slightly basic (Table 3a). This indicates that the formation of MeHg can occur downstream regardless of the amount of HgT if a methylating microorganism is present. Elemental analysis of the soil showed that most of the major elements in the soils were less than 1 % except for K, Al, Fe, and Si with varying concentrations (Table 4a, 4b, 4c, and 4d). Total carbon content was much greater in SB 5-8 (13.8%) compared to SB 14-8 (2.81%) and similarly, twice the amount of organic carbon in SB 5-8 (4.2%) versus 2.2% in SB 14-8 (Table 3c).

Particle size analysis showed that the soils are basically silty clay loam. Results of detailed characterization of the stream water are presented in Table 3a. Other measurements such as geographical location, Atterberg limits, sample collection time, and depths are shown in Table 5a, 5b, and 5c respectively.

Mercury Leachability under wet and dry conditions

Experimental results indicate that the amount of leachable Hg varies by orders of magnitude in the three soil samples. Soils from Hinds Creek, control site, leached 10 ng/L when the soils were held under wet conditions. The wet soils from EFPC sites SB 14-8 and SB 5-8 leached 1.0×10^3 ng/L and 1.0×10^4 ng/L respectively (Fig. 7). The concentration of Hg in the soils from SB 5-8 is approximately 100 times higher than those from SB 14-8. The results showed how the "leachable" Hg fraction (defined as the fraction of Hg in the soil which can be mobilized into water) changes when soils undergo wetting and drying cycles, which are common in nature.

Microbial Isolation and Bacterial Enumeration

Number of culturable microorganisms capable of thriving in Hg contaminated soils

containing bioavailable nutrients was determined using soil extracts. Our observation showed that this technique could be a very useful tool to detect environmental microorganisms without adding any chemical nutrient to boost the growth of the organisms, which provide optimal growth conditions for various organisms capable of growing in such an environment with high amount of Hg. DNA was isolated from pure strains of the various medium, and were sequenced using next generation sequencing. Different microbes were identified from the different medium. Some of the microbes identified include *Burkholderia* (98%) in all the three soil types, *Serratia marcescens* (99%) only in SB14-8, and *Acinetobacter rhizosphaerae* (99%) and *Acinetobacter others* (99%) in only SB5-8 (Fig. 4).

We have isolated and identified different microbes that can thrive in Hg concentrations; some with different pigmentations based on microscopy (Table 2a and Fig. 3), physiological and chemical characteristics. Potential organisms able to survive in Hg contaminated soils were isolated on both non-selective commercially prepared media and natural media prepared from the soil extracts. The organisms were also cultured on various selective media (Table 2a). Several studies have evaluated Hg resistance in different microorganisms by inoculating various amount of Hg to the culture medium followed by incubation [Száková et al 2016; François et al 2012]. When *S. marcescens* was directly introduced to soils with the sorbent, the sorbent absorbed more Hg from the soil compared to culture solution without soil (Fig. 5 and 8). A clear understanding that not all bacteria from a given environment will grow on all laboratory media [Xu et al 2015] is very important to further characterize microbial diversity in an environment. Using the Hg contaminated soils containing known amounts of Hg to develop a formulated agar for microbial growth, we observed that only the pigmented organism grew on the formulated agar after 72 hrs compared to when grown on the nutrient agar (growth rate of 24 hrs). From the results, the growth rate of the organisms on the different medium varied. After 24 hours of incubation, robust colonies were found on the rich nutrient agar: SB5-8 = 9.0×10^7, SB14-8 = 9.4×10^7 and HC = 8.9×10^7, but not on the agar containing the soil extract. However, colony formation on the plate containing soil extracts became more detectible after 72 hours. Organisms that were transferred from nutrient agar to the natural medium grew poorly with little or no colonies. This could be due to the Hg concentration, which did not vary in the soil samples. Also, the availability of nutrient was strictly the same as in the sampling environment with minimal to no modification.

Mercury Biosorption using Sorbents and Microorganism:

Organoclay complex was shown to have a great affinity with Hg in solution [Wang and Abraham 2009]. In our analysis, organoclay did not show any significant absorption of Hg compared to *S. marcescens* alone (Fig. 8), neither when both were combined. In the presence of nutrient media used for culturing there was no increase of HgT. Subsequently, *S. marcescens* showed more reduction of HgT.

Total DNA

The results after extraction showed that SB 5-8 gave highly concentrated DNA than that of Hind-1 or SB 14-8 (Fig. 2). Ethanol precipitation increased the quality yield of the gDNA and further aided in removing any bound organic matter that might inhibit chances for optimal DNA sequencing or binding during molecular studies like PCR.

CONCLUSIONS

Isolation and identification of environmental microorganisms is very challenging due to the heterogonous nature of soils [Kirk et al 2004]. Various strategies have been employed to isolate and understand the behavior of individual microorganism in the presence of increased soil Hg contents [Száková et al 2016; Frentiu et al 2013]. Growing organisms in its natural environment provides information in the selection of suitable organisms capable of surviving using bioavailable nutrients. In addition, pH also plays a very crucial role for the optimum growth of bacteria.

S. marcescens has been identified to significantly reduce HgT [Cui et al 2011]; however, little is known on its role in Hg speciation. It does contribute to the conversion of organomercuric and mercuric Hg to volatile Hg through intracellular transformation linked to the expression of mercury resistance (*mer*)S genes [Reinfeldera et al 1998]. The pathway of Hg methylation in microorganism have not been fully understood nor the specific mechanism that *S. marcescens* uses to reduce Hg. On the other hand, Hg transformation in *S. marcescens* could be associated with substrate specificity of its enzymes [Gilmour et al 2013]. Our result using spiked Hg showed that *S. marcescens* can absorb 50% of Hg^{2+} (Fig. 8). However, when inoculated in the contaminated soil, sorbent (Organoclay$^{PM\ 199}$) showed more HgT reduction compared to *S. marcescens* alone (Fig. 5). However, their performance in an environmental condition with other organisms needs to be evaluated. Unlike other *S. marcescens* isolates used in the previous Hg studies, ours was a pure culture isolated from a Hg contaminated site that is currently being studied. This strain has been shown to withstand rigorous environmental conditions and can easily be cultured in laboratory medium.

Sorbent Organoclay complexes have been tested to effectively reduce HgT in solution, but have not been evaluated for its efficacy in the presence of microorganisms. Consequently, we performed a study to examine the influence of microorganisms on the efficacy of HgT reduction by the sorbents. Our results did not show any significant influence of *S. marcescens* on the sorbents compared to *S. marcescens* alone, even though *S. marcescens* was isolated from the contaminated sites. Therefore, a clear understanding of its role or influence in reducing HgT in contaminated soils in the presence of sorbents and other microbes needs to be further evaluated because *S. marcescens* can form biofilms in the presence of other microbes.

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REFERENCES

Barkay, T., Miller, S. M., & Summers, A. O. (2003). Bacterial mercury resistance from atoms to ecosystems. *FEMS microbiology reviews*, *27*(2-3), 355-384.

Chen, X., Feng, X., Liu, J., Fryxell, G. E., & Gong, M. (1999). Mercury separation and immobilization using self-assembled monolayers on mesoporous supports (SAMMS). *Separation science and technology*, *34*(6-7), 1121-1132.

Cui, W. W., Wang, X. L., Duan, J. J., Yang, M., Zi, W., & Liu, X. C. (2011). Advances on remediation techniques of heavy metal Cd and Hg in contaminated soil. *Guizhou Agric Sci*, *7*, 67.

Ehrlich, H. L. (1997). Microbes and metals. *Applied microbiology and biotechnology*, *48*(6), 687-692.

François, F., Lombard, C., Guigner, J. M., Soreau, P., Brian-Jaisson, F., Martino, G., ... & Peduzzi, J. (2012). Isolation and characterization of environmental bacteria capable of extracellular biosorption of mercury. *Applied and environmental microbiology*, *78*(4), 1097-1106.

Frentiu, T., Pintican, B. P., Butaciu, S., Mihaltan, A. I., Ponta, M., & Frentiu, M. (2013). Determination, speciation and distribution of mercury in soil in the surroundings of a former chlor-alkali plant: assessment of sequential extraction procedure and analytical technique. *Chemistry central journal*, *7*(1), 178.

Gilmour, C. C., Podar, M., Bullock, A. L., Graham, A. M., Brown, S. D., Somenahally, A. C., ... & Elias, D. A. (2013). Mercury methylation by novel microorganisms from new environments. *Environmental science & technology*, *47*(20), 11810-11820.

Gilmour, C. C., Elias, D. A., Kucken, A. M., Brown, S. D., Palumbo, A. V., Schadt, C. W., & Wall, J. D. (2011). Sulfate-reducing bacterium Desulfovibrio desulfuricans ND132 as a model for understanding bacterial mercury methylation. *Applied and environmental microbiology*, *77*(12), 3938-3951.

He, F., Liang, L., & Miller, C. (2010). Technology evaluation for waterborne mercury removal at the Y-12 National Security Complex. *Oak Ridge*.

Hsu-Kim, H., Kucharzyk, K. H., Zhang, T., & Deshusses, M. A. (2013). Mechanisms regulating mercury bioavailability for methylating microorganisms in the aquatic environment: a critical review. *Environmental science & technology*, *47*(6), 2441-2456.

Jeremiason, J. D., Engstrom, D. R., Swain, E. B., Nater, E. A., Johnson, B. M., Almendinger, J. E., ... & Kolka, R. K. (2006). Sulfate addition increases methylmercury production in an experimental wetland. *Environmental science & technology*, *40*(12), 3800-3806.

Kirk, J. L., Beaudette, L. A., Hart, M., Moutoglis, P., Klironomos, J. N., Lee, H., & Trevors, J. T. (2004). Methods of studying soil microbial diversity. *Journal of microbiological methods*, *58*(2), 169-188.

Lee, S. W., Lowry, G. V., & Hsu-Kim, H. (2016). Biogeochemical transformations of mercury in solid waste landfills and pathways for release. *Environmental science: processes & impacts*, *18*(2), 176-189.

Lin, C. C., Yee, N., & Barkay, T. (2012). Microbial transformations in the mercury cycle. *Environmental chemistry and toxicology of mercury*, 155-191.

Marvin-DiPasquale, M., Windham-Myers, L., Agee, J. L., Kakouros, E., Kieu, L. H., Fleck, J. A., ... & Stricker, C. A. (2014). Methylmercury production in sediment from agricultural and non-agricultural wetlands in the Yolo Bypass, California, USA. *Science of the total environment*, *484*, 288-299.

Nwuche, C. O., & Ugoji, E. O. (2008). Effects of heavy metal pollution on the soil microbial activity. *International journal of environmental science & technology*, *5*(3), 409-414.

Pirrone, N., Cinnirella, S., Feng, X., Finkelman, R. B., Friedli, H. R., Leaner, J., ... & Telmer, K. (2010). Global mercury emissions to the atmosphere from anthropogenic and natural sources. *Atmospheric chemistry and physics*, *10*(13), 5951-5964.

Podar, M., Gilmour, C. C., Brandt, C. C., Soren, A., Brown, S. D., Crable, B. R., ... & Elias, D. A. (2015). Global prevalence and distribution of genes and microorganisms involved in mercury methylation. *Science advances*, *1*(9), e1500675.

Reinfeldera, J. R., Fisherb, N. S., Luomac, S. N., Nicholsd, J. W., & Wange, W. X. (1998). Trace element trophic transfer in aquatic organisms: A critique of the kinetic model approach. *The science of the total environment*, *219*, 117135.

Robinson, J. B., & Tuovinen, O. H. (1984). Mechanisms of microbial resistance and detoxification of mercury and organomercury compounds: physiological, biochemical, and genetic analyses. *Microbiological reviews*, *48*(2), 95.

Sobolev, D., & Begonia, M. (2008). Effects of heavy metal contamination upon soil microbes: lead-induced changes in general and denitrifying microbial communities as evidenced by molecular markers. *International journal of environmental research and public health*, *5*(5), 450-456.

Sterritt R. M., &, J. Lester N. (1980). Interactions of heavy metals with bacteria. *Science of the total environment*, *14*(1), 5-17.

Száková, J., Havlíčková, J., Šípková, A., Gabriel, J., Švec, K., Baldrian, P., ... & Komárek, J. (2016). Effects of the soil microbial community on mobile proportions and speciation of mercury (Hg) in contaminated soil. *Journal of environmental science and health, part A*, *51*(4), 364-370.

Tottey, S., Harvie, D. R., & Robinson, N. J. (2007). Understanding how cells allocate metals. *Molecular microbiology of heavy metals,* 3-35. Springer Berlin Heidelberg.

Wang, Z., & Abraham, R. (2009). *U.S. Patent No. 7,510,992*. Washington, DC: U.S. Patent and Trademark Office.

Xu, J., Bravo, A. G., Lagerkvist, A., Bertilsson, S., Sjöblom, R., & Kumpiene, J. (2015). Sources and remediation techniques for mercury contaminated soil. *Environment international*, *74*, 42-53.

Yu, R. Q., Reinfelder, J. R., Hines, M. E., & Barkay, T. (2013). Mercury methylation by the methanogen Methanospirillum hungatei. *Applied and environmental microbiology*, *79*(20), 6325-6330.

Yu, R. Q., Flanders, J. R., Mack, E. E., Turner, R., Mirza, M. B., & Barkay, T. (2012). Contribution of coexisting sulfate and iron reducing bacteria to methylmercury production in freshwater river sediments. *Environmental science & technology*, *46*(5), 2684-2691.

Fig. 1: East Fork Popular Creek where the soil was sampled from the bank of the creek.

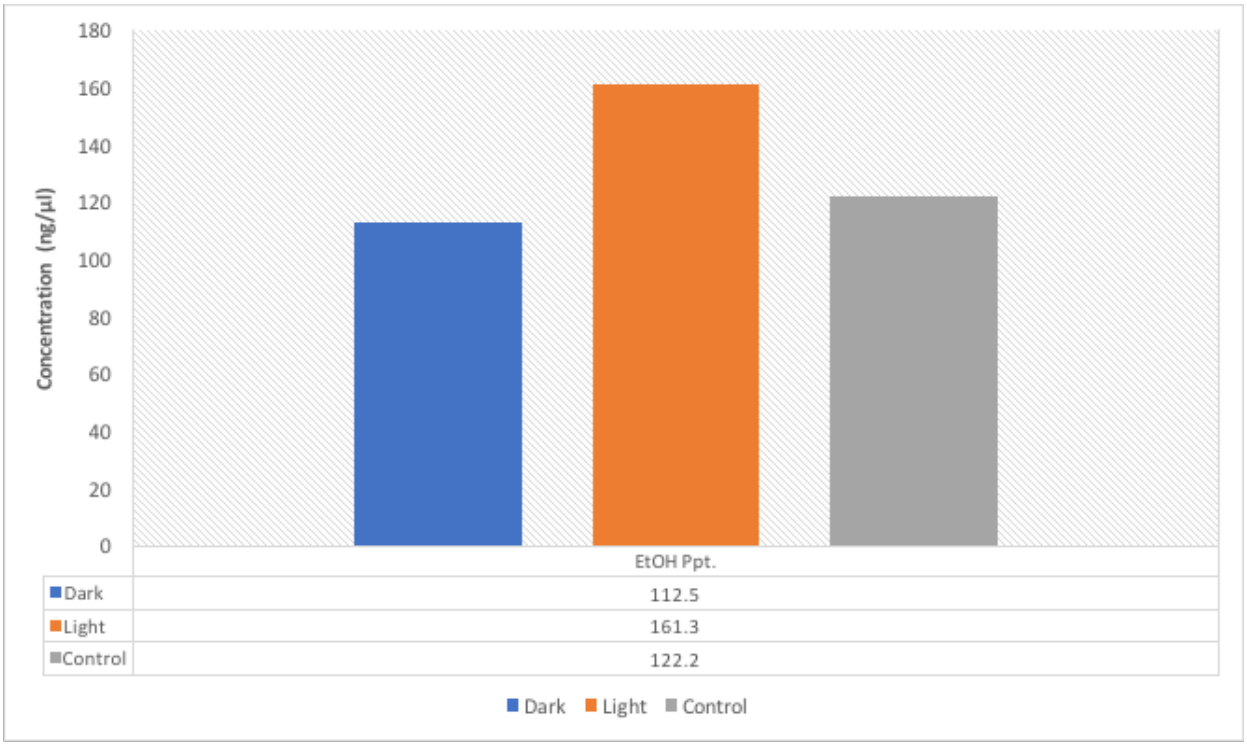

Fig. 2: Graph showing Soil DNA concentration

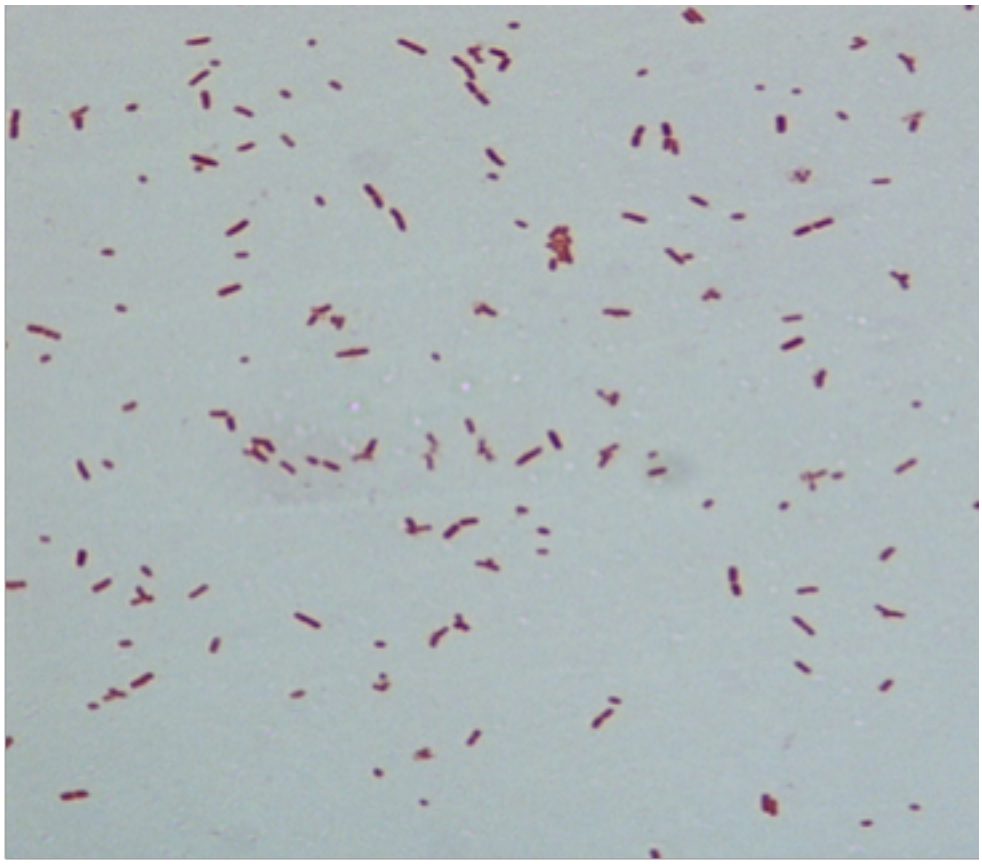

Fig. 3: Electron micrograph showing the morphology of *S. marcescens* gram staining.

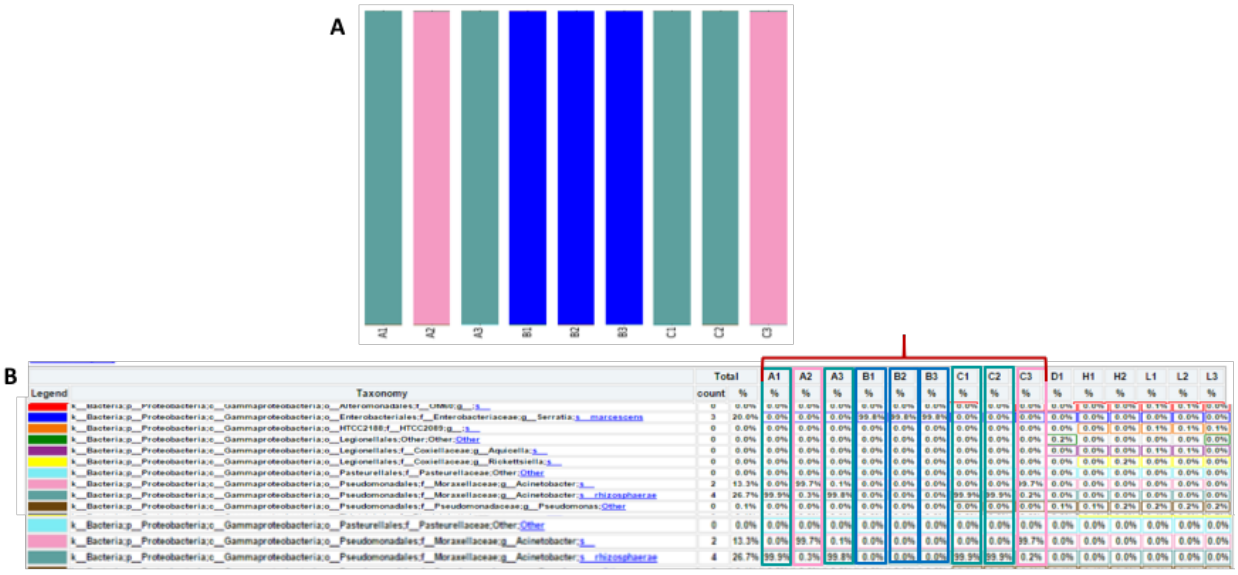

Fig. 4: Taxonomic analysis of pure cultures isolated from soils. Isolated microbes were identified to belong to the Proteobacteria phylum. (A). Soil Types: A= SB 14-8; B= SB 5-8 and C= Hind-1. (B). Taxonomic legend showing the percentage of resemblance of the different isolated organisms.

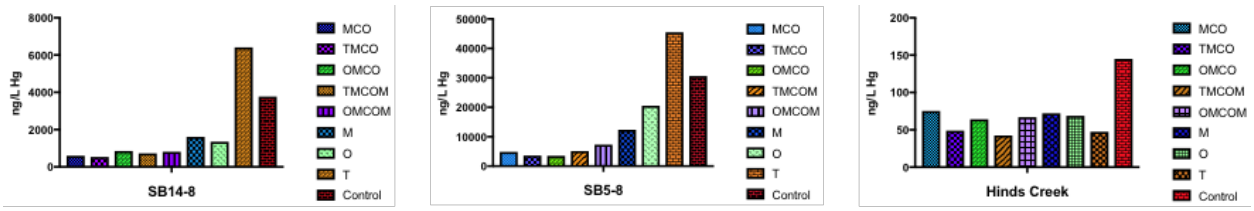

Fig. 5: MCO: *S. marcescens;* T: Thiol SAMMS; O: Organoclay $^{PM-199}$; TMCO: Thiol-SAMMS and *S. marcescens;* OMCO: Organoclay $^{PM-199}$ and *S. marcescens;* M: Nonselective nutrient broth; TMCOM: Thiol-SAMMS, *S. marcescens* and nonselective nutrient broth; OMCOM: Organoclay $^{PM-199}$, *S. marcescens* and nonselective nutrient broth. Comparing the amount of Hg that can be sorbed by *S. marcescens* compared to sorbents or combined

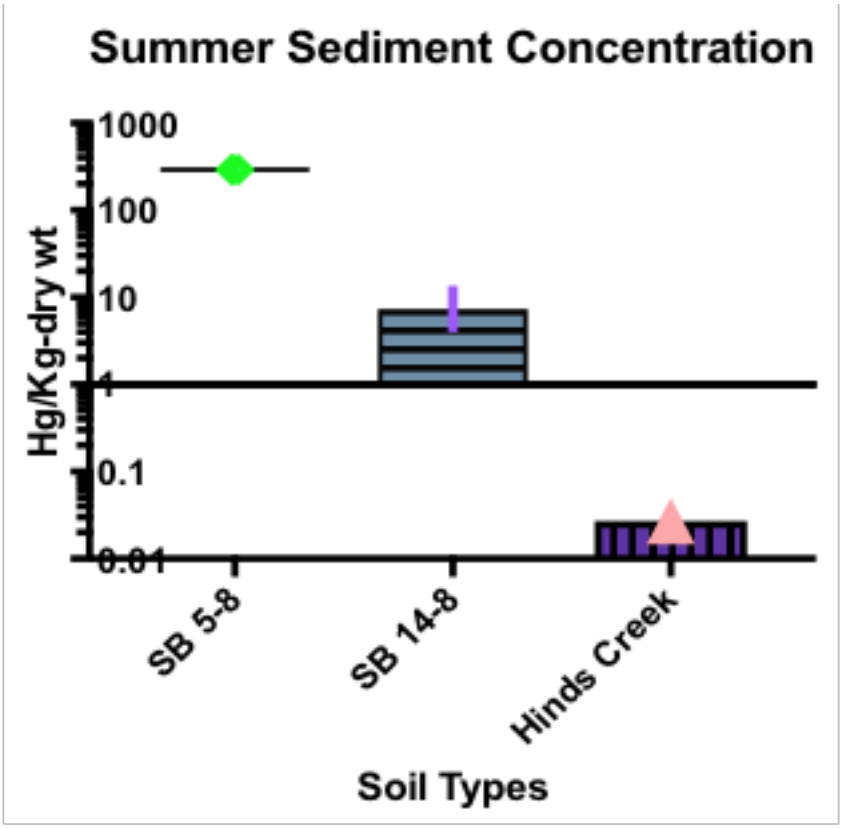

Fig. 6: Total mercury concentration in soils.

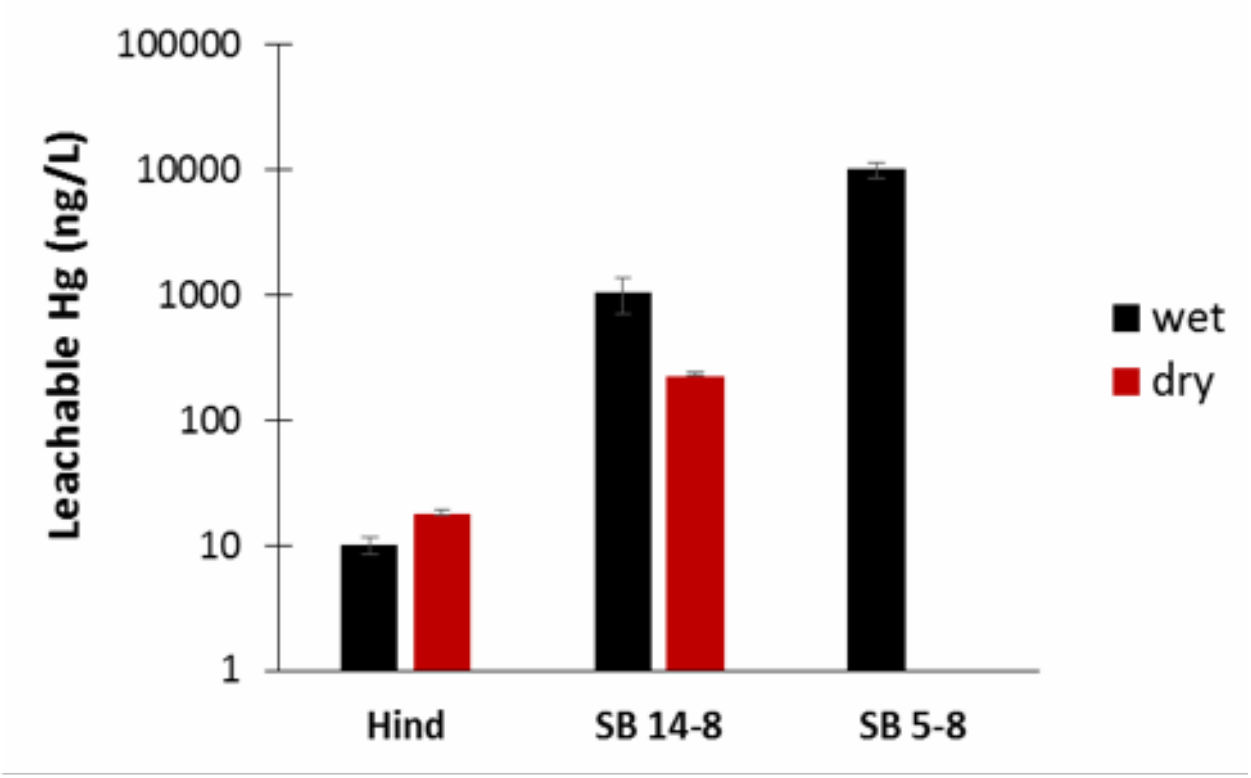

Fig. 7: Leachable Hg in soils under wet and dry conditions.

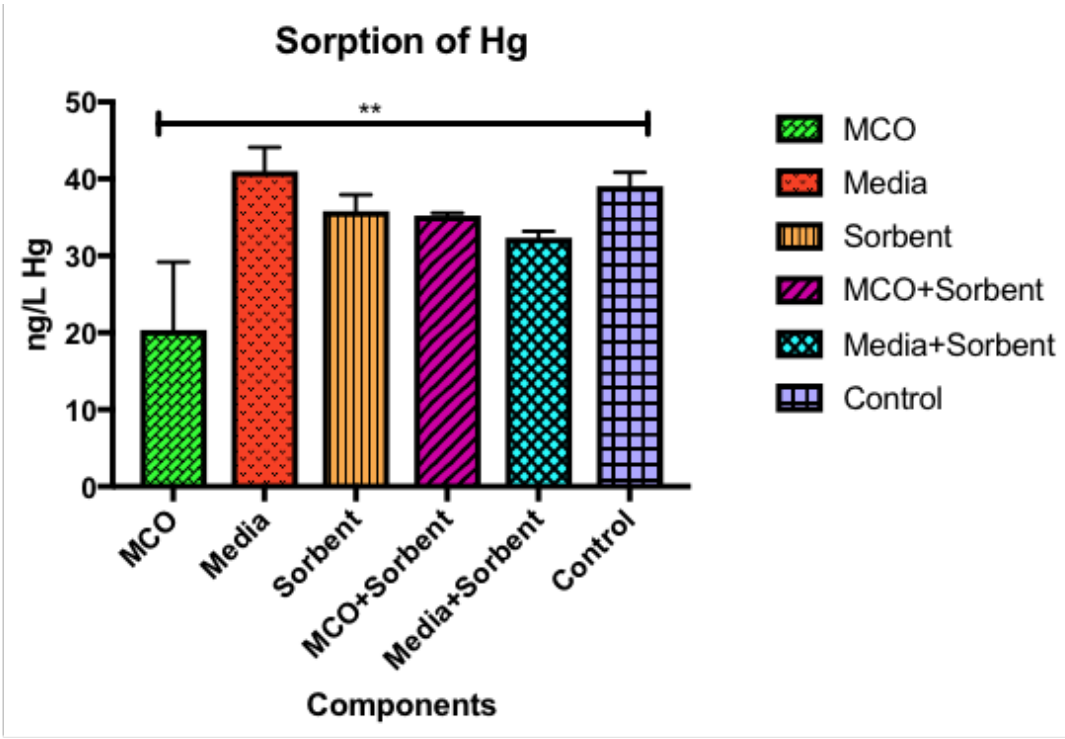

Fig. 8: Recovery of Hg^{2+} after incubating *S. marcescens* with culture media and sorbents. MCO: *S. marcescens*; Sorbent: Thiol SAMMS. Comparing the amount of Hg that can be sorbed by *S. marcescens* compared to sorbents or combined. There was a significant difference in the amount of Hg sorbed by *S. marcescens* compared to the sorbent or combined means. P value 0.0012**. Error bars represents the standard deviation of triplicates.

Table 1:

Sorbent	Description	Source
Thiol-SAMMS®	Thiol-functionalized self-assembled monolayer on mesoporous silica support	**Steward Environmental Solutions, LLC**
Organoclay™ PM-199	**Functionalized bentonite-based clay**	**CETCO**

Overview of sorbent materials evaluated.

Table 2:

Soil Samples	A	Nutrient Agar		B	Soil Extract	
	Red	Milky Lighter	Milky Darker	24	48	72
SB 5-8	+	++	+++	+	+	++
SB 14-8	+	+++	+++	-	+	++
Hind Creek	-	+++	+++	-	++	+++

+ = growth
- = no growth

(A) Isolation of bacteria on non-selective nutrient media and (B) Soil extracts or natural media.

Table 3:

A

Sample ID	pH	
	5 mM $CaCl_2$	DIW
SB 5-8	7.809	8.244
SB 14-8	7.474	8.006
Hinds Creek	N/A	N/A

B

Sample ID	Stream Water				
	Temp	PH	ORP	Conductivity	Vegetation
SB 5-8	16.4 C	8.21	324 mV	317.1 µS/cm	Absent
SB 14-8	17.4 C	8.30	335 mV	364.3 µS/cm	Present
Hinds Creek	17.6 C	8.37	320 mV	349.9 µS/cm	Absent

C

Analyte Symbol	C-Total	C-Organic (calc)	TKN
Unit Symbol	%	%	%
SB 5-8	13.8	4.2	0.4
SB 14.8	2.81	2.2	0.2

(A) Analysis of the physical characteristics of soils (B) East Fork Popular Creek where the soils were sampled (C) Organic carbon composition of the soil.

Table 4:

4a

Analyte Symbol	As	Al	Be	Co	Ca
Unit Symbol	%	%	%	%	%
SB 5-8	< 0.01	7.15	< 0.001	0.002	0.90
SB 14.8	< 0.01	3.8	< 0.001	< 0.002	0.32

4b

Analyte Symbol	Cr	Cu	Fe	Li	K
Unit Symbol	%	%	%	%	%
SB 5-8	< 0.01	0.019	3.49	0.013	1.60
SB 14.8	< 0.01	< 0.005	1.97	0.003	1.60

4c

Analyte Symbol	Mg	Mn	Ni	Pb	P
Unit Symbol	%	%	%	%	%
SB 5-8	0.56	0.20	0.02	0.01	0.106
SB 14.8	0.33	0.11	< 0.005	< 0.01	0.070

4d

Analyte Symbol	S	Si	Ti	W	Zn
Unit Symbol	%	%	%	%	%
SB 5-8	0.12	23.3	0.50	< 0.005	0.02
SB 14.8	0.04	34.5	0.45	< 0.005	0.01

(4a –d) Soils chemical compositions.

Table 5:

A

Sample ID	EFK	Latitude	Longitude
SB 5-8	18.43	36.00438	**-84.28246**
SB 14-8	10.65	35.97514	**-84.33739**
Hinds Creek	**N/A**	**36.14073**	**-84.05129**

B

Atterberg Limits					
Sample ID	PctMoist	PlastIndex	LiqLimit	PlasticLim	USCS
SB 5-8	27.67	19.0	40.7	21.7	CL
SB 14-8	27.28	14.9	37.0	22.1	CL
Hinds Creek	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**

C

Sample ID	EFK	Collection time	Sample depths
SB 5-8	18.43	10.45 AM	**26"**
SB 14-8	10.65	11:20 AM	**26"**
Hinds Creek	**N/A**	**12:45 PM**	**24"**

(A) Soils physical characteristics measured; (B) Geographical location where soils were collected; (C) Information on soil collection time, depths and acidity level. Depths are in inches below ground surface; DIW is distilled water.