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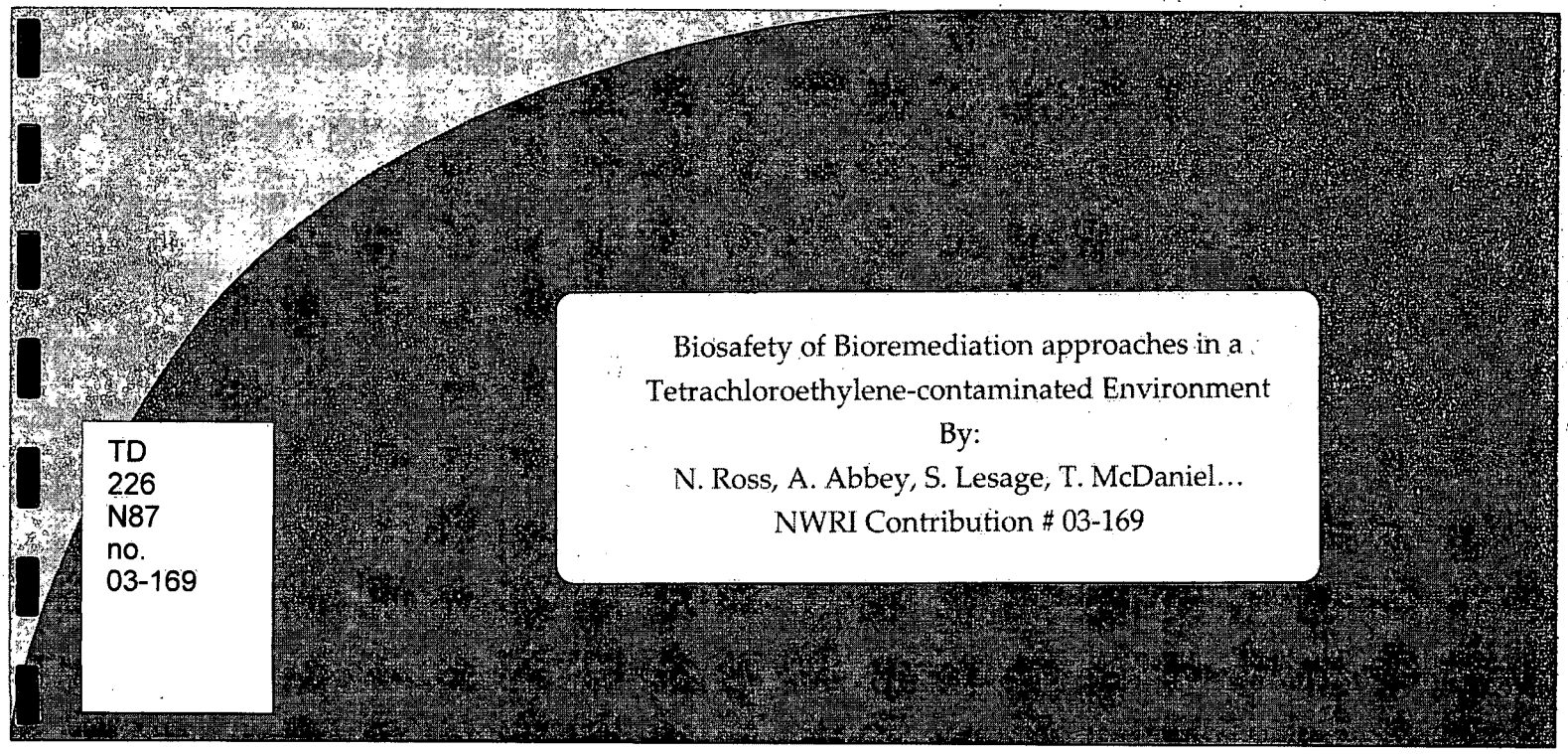

Biosafety of Bioremediation approaches in a Tetrachloroethylene-contaminated Environment

By:
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03-169

BIOSAFETY OF BIOREMEDIATION APPROACHES IN A TETRACHLOROETHYLENE-CONTAMINATED ENVIRONMENT

Nathalie Ross, Ann-Marie Abbey, Suzanne Lesage, Tana McDaniel,
Pamela Martin, Elizabeth Edwards and David Major

ABSTRACT: To assess whether the injection of an adapted culture in groundwater (bioaugmentation) is as biosafe as stimulating the indigenous bacteria (biostimulation) or observing natural processes (natural attenuation), a large-scale aquifer (6.0 × 2.4 × 1.8 m) was divided in three lanes for a comparative study in a tetrachloroethylene(PCE)-contaminated groundwater assessing the biodegradation products, the fate of injected and indigenous bacteria, and a battery of biotests. Selected results from the first 250 days confirmed that bioaugmentation was effective for reductive dechlorination to ethene, whereas *cis*-DCE remained in the effluent from the biostimulation lane and no degradation was measured in the natural attenuation lane. The bacterial density was consistent over time and space in the model aquifer, but the partitioning, 5 log · mL^{-1} in the groundwater and 12 log · g^{-1} on the sand particles, suggested the active population to be sessile. As a potential receptor of groundwater through resurgence, a model amphibian was chronically exposed to effluents from the three lanes; although the froglets had a significant higher weight compared to the controls, the survivorship and metamorphic transformation were not significantly affected by the effluents. This information will be used to help define regulatory requirements for *in situ* bioremediation approaches.

NWRI RESEARCH SUMMARY

Plain language title
Biosafety of bioremediation approaches in a tetrachloroethylene-contaminated environment

What is the problem and what do sicentists already know about it?
The application of bioremediation techniques is subject to environmental regulations requiring the provision of information on the environmental fate and ecological effects of injected microorganisms.

Why did NWRI do this study?
NWRI has the expertise on microbiology and ecotoxicology assessment. The large-scale model aquifer, in AQUEREF at NWRI, was an ideal setting to conduct such a comparative biotechnological study.

What were the results?
Bioaugmentation, the addition of adpated bacteria to degrade a targeted contaminant, was effective to transform a fraction of PCE into ethene whereas intermediate compounds were found where biostimulation, the addition of nutrients to stimulate the indigenous microbial population, was applied in the model aquifer. Enumeration of bacteria in groundwater and on the soil particles suggested that the active population is attached to the soil. As a potential receptor of groundwater, a model amphibian was chronically exposed to effluents; although the froglets had a significant higher weight compared to the controls, the survivorship and metamorphic transformation were not significantly affected by the effluents. Bioaugmentation is effective to biodegrade PCE to harmless compounds, and no significant toxicity was measured from the effluent.

How will these results be used?
This information will be used to help define regulatory requirements for in situ bioremediation approaches.

Who were our main partners in the study?
Canadian Wildlife Services, University of Toronto, and GeoSyntec Consultants

BIOSÉCURITÉ DES MÉTHODES DE BIORESTAURATION DANS UN ENVIRONNEMENT CONTAMINÉ PAR LE TÉTRACHLOROÉTHYLÈNE

Nathalie Ross, Ann-Marie Abbey, Suzanne Lesage, Tana McDaniel,
Pamela Martin, Elizabeth Edwards et David Major

RÉSUMÉ - Pour déterminer si l'injection d'une culture adaptée dans l'eau souterraine (bioaugmentation) offre une biosécurité aussi stimulante que les bactéries indigènes (biostimulation) ou pour observer les processus naturels (atténuation naturelle), un aquifère à grande échelle (6,0 × 2,4 × 1,8 m) a été divisé en trois bandes pour une étude comparative dans une eau souterraine contaminée par le tétrachloroéthylène (PCE), aux fins de l'évaluation des produits de biodégradation, du devenir des bactéries injectées et indigènes, et enfin de la réalisation d'une batterie de bioessais. Les résultats sélectionnés des 250 premiers jours ont confirmé que la bioaugmentation permettait une déchloration réductive efficace en éthène, alors que le *cis*-DCE demeurait dans l'effluent provenant de la bande avec biostimulation et qu'aucune dégradation n'a été mesurée dans la bande avec atténuation naturelle. La densité bactérienne était régulière dans le temps et l'espace à l'intérieur de l'aquifère modèle, mais le partage, 5 log · mL^{-1} dans l'eau souterraine et 12 log · g^{-1} sur les particules de sable, semblait montrer que la population active est sessile. En tant que récepteur potentiel de l'eau souterraine lors de sa résurgence, un amphibien modèle a été exposé chroniquement aux effluents provenant des trois bandes; en dépit du fait que les petites grenouilles avaient un poids sensiblement plus élevé que les témoins, la survie et la transformation métamorphique ne se trouvaient pas altérées de façon significative par les effluents. Cette information aidera à définir les exigences en matière de réglementation pour les méthodes de biorestauration *in situ*.

Sommaire des recherches de l'INRE

Titre en langage clair
La biosécurité des méthodes de biorestauration dans un milieu contaminé par le tétrachloroéthylène.

Quel est le problème et que savent les chercheurs à ce sujet?
L'application des techniques de biorestauration est soumise à la réglementation environnementale exigeant la communication de renseignements sur le devenir environnemental et les effets écologiques des microorganismes injectés.

Pourquoi l'INRE a-t-il effectué cette étude?
L'INRE possède de l'expertise en évaluation microbiologique et écotoxicologique. L'aquifère modèle à grande échelle, à l'AQUEREF de l'INRE, constituait un cadre idéal pour réaliser une étude biotechnologique comparative de ce type.

Quels sont les résultats?
La bioaugmentation, addition de bactéries adaptées pour dégrader un contaminant ciblé, a permis de transformer efficacement une fraction du PCE en éthène, alors qu'il y avait présence de composés intermédiaires dans le cas de l'application de la biostimulation à l'aquifère modèle, soit l'addition de nutriments pour stimuler la population microbienne indigène. Le dénombrement des bactéries dans l'eau souterraine et sur les particules de sol semble montrer que la population active est fixée au sol. En tant que récepteur potentiel de l'eau souterraine, un amphibien modèle a été exposé chroniquement aux effluents; en dépit du fait que les petites grenouilles avaient un poids sensiblement plus élevé que les témoins, la survie et la transformation métamorphique ne se trouvaient pas altérées de façon significative par les effluents. La bioaugmentation permet de biodégrader efficacement le PCE en composés inoffensifs; aucune toxicité significative n'a été mesurée en provenance de l'effluent.

Comment ces résultats seront-ils utilisés?
Cette information servira à établir les exigences réglementaires pour les méthodes de biorestauration in situ.

Quels étaient nos principaux partenaires dans cette étude?
Service canadien de la faune, Université de Toronto, GeoSyntec Consultants

BIOSAFETY OF BIOREMEDIATION APPROACHES IN A TETRACHLOROETHYLENE-CONTAMINATED ENVIRONMENT

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Tana McDaniel and Pamela Martin (Canadian Wildlife Service, Environment Canada, Burlington, Ontario, Canada)
Elizabeth Edwards (University of Toronto, Toronto, Ontario, Canada)
David Major (GeoSyntec Consultants, Guelph, Ontario, Canada)

ABSTRACT: To assess whether the injection of an adapted culture in groundwater (bioaugmentation) is as biosafe as stimulating the indigenous bacteria (biostimulation) or observing natural processes (natural attenuation), a large-scale aquifer (6.0 × 2.4 × 1.8 m) was divided in three lanes for a comparative study in a tetrachloroethylene(PCE)-contaminated groundwater assessing the biodegradation products, the fate of injected and indigenous bacteria, and a battery of biotests. Selected results from the first 250 days confirmed that bioaugmentation was effective for reductive dechlorination to ethene, whereas *cis*-DCE remained in the effluent from the biostimulation lane and no degradation was measured in the natural attenuation lane. The bacterial density was consistent over time and space in the model aquifer, but the partitioning, 5 log · mL^{-1} in the groundwater and 12 log · g^{-1} on the sand particles, suggested the active population to be sessile. As a potential receptor of groundwater through resurgence, a model amphibian was chronically exposed to effluents from the three lanes; although the froglets had a significant higher weight compared to the controls, the survivorship and metamorphic transformation were not significantly affected by the effluents. This information will be used to help define regulatory requirements for *in situ* bioremediation approaches.

INTRODUCTION

The application of bioremediation techniques is subjected to environmental regulations particularly in providing governmental agencies with information on the environmental fate and ecological effects of injected microorganisms (Environment Canada, 1997; United States Environmental Protection Agency, 1997). To assess the fate of injected bacteria, biomolecular techniques are successfully used (van Elsas et al., 1998), but further scientific evidence has to be provided for the application of these regulations regarding bioremediation activities.

As information requested in respect to the ecological effects of the injected microorganisms, receptor species likely to be exposed should be included in the battery of biotest. As such, amphibians are an important component of wetland ecosystems and may be receptors through groundwater recharges. Limited information is available on the toxicity of chloroethylenes to amphibians; however, early embryonic exposures with TCE have shown later teratogenic damage to developing amphibian larvae (Fort et al., 1993).

Bioaugmentation has been shown to be effective to remediate groundwater contaminated with chlorinated products (Major et al., 2002), but the biosafety of this approach, as required by recent environmental regulations, has to be demonstrated. The present study—comparing three bioremediation approaches: natural attenuation (NA),

biostimulation (ST), and bioaugmentation (AU)—combines the monitoring of volatile organic carbons (VOCs), the assessment of the fate of injected bacteria, and the measurement of ecological effects. The selected results reported herein summarizes the first 250 days of this ongoing, multidisciplinary study.

MATERIALS AND METHODS

The Model Aquifer. The model aquifer consisted of a 6-m long, 2.4-m wide, and 1.8-m deep stainless-steel tank, divided in three 0.6-m lanes, and filled with clean, medium-to-fine grain sand (FIGURE 1). Groundwater, pumped on-site, was introduced into three head tanks, and the flow was maintained gravimetrically at 80 mL min^{-1}. Each lane was equipped with 66 sampling ports distributed along three depths and nine longitudinal transects, a PCE-source well, three injection wells, and a withdrawal well. As a control treatment, natural attenuation (NA) was compared to biostimulation (ST) (injection of methanol and lactic acid twice weekly) and bioaugmentation (AU) (injection of nutrients plus a single injection of the KB-1 culture) (Major et al., 2002).

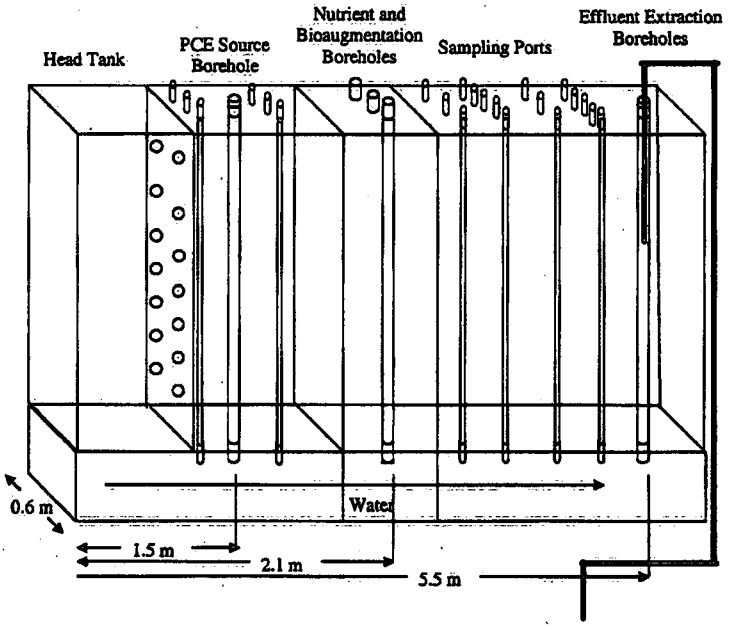

FIGURE 1. Cross-sectional view of one lane of the model aquifer

The PCE source consisted of PCE in silicone oil mixed with coarse sand (10 % w/w) introduced in a 30-cm, 200-μm meshed sock inserted at 1 m deep. The nomenclature used for the sampling ports was as follows: lane (NA, ST, or AU) , length from the head tank (ft), width from the center of the lane (A and E = 20 cm, B and D = 10 cm, and C = 0 cm), depth (ft) (ex. AU11D3).

VOCs and Microbiological Analyses. Groundwater samples were analyzed for VOCs by purge-and-trap GC/MSD (HP 5890 gas chromatograph/5973 mass spectrometer equipped with a DB-624 column) following USEPA methods 5030B/8260B. Because of

the high concentrations of the contaminants, they were diluted by as much as 4300 fold by using 100 μL of sample in a 43-mL VOC vial.

Groundwater was sampled through ports located downgradient of the wells in each of the three lanes in June and September 2002. Samples were refrigerated at 4°C until analyzed, within a 24-h time frame. A *Bac*Light™ viability test was performed as outlined by (Boulos et al., 1999).

A soil core was removed from each of the three lanes in July 2002. Soil collected was mixed to form a composite sample and analysis for cell density using *Bac*Light™ enumeration. One gram of the soil was added to a mixture of sterile 9.5 ml sterile 0.1 % sodium pyrophosphate buffer (pH 7.0) and 3 g glass beads (Leung et al., 1997) in a 50 ml Erlenmeyer flask. The slurry was shaken at 150 rpm at room temperature for 45 min to separate bacterial cells from the soil particles (Van Dyke et al., 1996). Serial dilutions of the slurry were prepared by adding 1 ml of the slurry to 9 ml of the sterile sodium pyrophosphate. A BacLight™ viability test was then performed on the dilutions using the same methods as listed above with the groundwater samples.

Chronic Exposures of the Aquifer Effluents to Amphibian Embryos. *Xenopus* tadpoles and embryos were exposed to 25 % effluent from each of the three lanes diluted with ~~in~~ filtered, dechlorinated tap water. Controls were exposed to groundwater from the head tank diluted in filtered tap water. Three replicates of 30 individuals were studied. Exposures were initiated on embryos less than 24-h old and continued for 100 days or until individuals reached metamorphosis. When *Xenopus* reached metamorphic transformation, they were euthanized and weighed, and snout vent length (SVL) ~~were~~ was measured.

RESULTS AND DISCUSSION

Fate of VOCs in the Model Aquifer. The evolution of the VOC concentrations in the three treatment lanes and the effluents are summarized on FIGURE 2. The data at monitoring points 4C4 demonstrate the dissolution of the PCE from the source, prior to the addition of ST or AU. The fluctuation in the data reflects the source heterogeneity and possible escapes of DNAPL blobs from the source, but overall, the three different sources were relatively well replicated and provided an average input concentration of 200 μmoles/L for the first 50 days, then started to decline exponentially. However, the source in the AU lane did not last as long as the ~~low~~ other lanes. The effluent concentrations peaked simultaneously in the 3 lanes at about 100 days before declining as well. It is shortly after that degradation products started to appear in the effluents.

The distribution of degradation products are shown at sampling point 15C4 (or 15C5) for each lane on FIGURE 3 a-c. As anticipated, no degradation products were observed in the NA lane (a). In the ST lane (b), *cis*-DCE, started to appear after 150 days, TCE after 200 days, and only in the AU lane were there any VC or ethene measured. Significant amounts of methane (200 to 400 μmoles/L) were generated in both the ST and AU lanes, but none was found the NA lane.

There was no significant difference in the total amount of VOCs in the effluent of each treatment. It is not entirely surprising that the different treatments did not have any effect on the total amount of VOCs because it was added after the source. Therefore, any biosurfactant formed would not be in contact with the source.

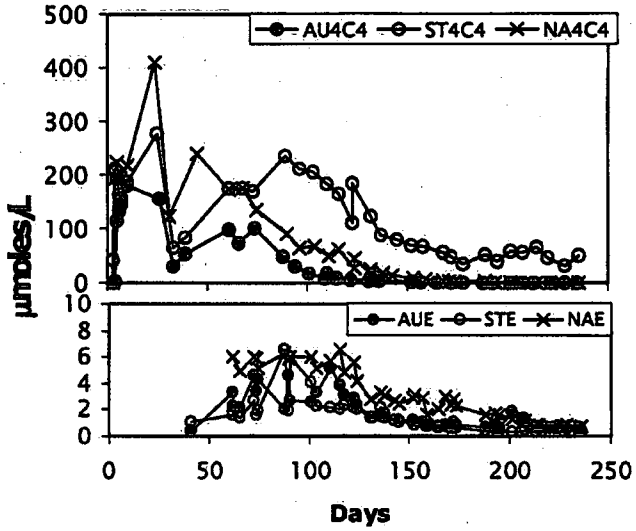

FIGURE 2. Source dissolution in the three treatments and the effluents concentrations. The source contained 10% tetrachloroethylene and 90% silicone oil.

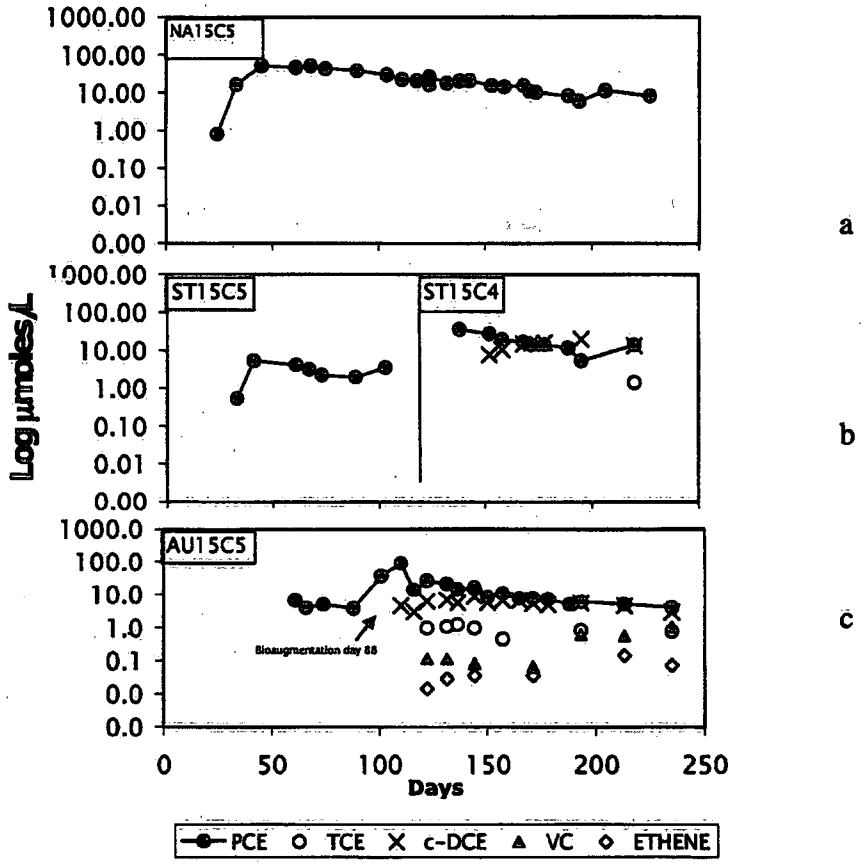

FIGURE 3. Degradation products observed in each lane. Note that the y axis is in logs in order to see all degradation products.

Microbiological Monitoring. The bacterial density in groundwater was consistent over time and space (TABLE 1). The bacterial density attached to the soil was 7 orders of magnitude higher than in suspension in the groundwater, which can be an indication of the majority of active bacteria being attached (van Schie and Fletcher, 1999). Therefore, the partitioning of the bioaugmentation culture, KB-1 isolated from a TCE-contaminated site, might be preferentially attached to the soil particles.

TABLE 1. Enumeration of total bacteria using BacLight™.

Sample	Groundwater (log total bacteria / ml)		Soil (Log total bacteria / g)
	June 2002	September 2002	July 2002
Holding tank		5.25	
NA6C4	5.53		
NA8C4	5.65		
NA15C5	5.37	5.23	
NA-soil composite			12.01
ST15C5		5.43	
ST-soil composite			12.06
AU15C5		5.01	
AU-soil composite			11.71

Additional microbiological analyses, such as DGGE and monitoring KB-1 with biomolecular probes, will give insights into the fate of the bioaugmented bacterial population in the model aquifer.

Assessment of the Impacts of Bioremediation using Amphibian Larvae. There was no significant difference in the survivorship between the control group and those exposed to any of the three treatments ($p > 0.05$, TABLE 2).

TABLE 2. End points measured on *Xenopus* tadpoles exposed to effluents from the model aquifer.

Treatment	Survivorship t = 68 d (%)	Survivorship t = 100 d (%)	Transformed (%)	Weight (g)	Snout Vent Length (mm)
NA	66.3 ±17.4	70.0 ±20.3	38.4 ±14.8	0.44 ±0.20	16.12 ±0.27
ST	66.7 ±27.8	65.4 ±25.0	48.1 ±14.6	0.39 ±0.20	15.84 ±0.23
AU	60.6 ±5.8	58.4 ±4.3	52.3 ±11.5	0.44 ±0.20	13.26 ±0.21
Control	88.3 ±1.4	70.2 ±11.4	32.0 ±14.0	0.36 ±0.20	15.23 ±0.20

Of the survivors, there was no significant difference in the proportion of individuals to reach metamorphic transformation within the 100-d period. There was a significant difference in weight and SVL of *Xenopus* froglets with controls being smaller than animals exposed to the three effluents. However, no obvious bacterially induced lesions were observed in tadpoles or transformed froglets.

CONCLUSIONS

Bioaugmentation does generate complete dechlorination product, whereas biostimulation enhances existing population, which very often lack the ability to degrade PCE beyond *cis*-DCE. In the absence of intervention, no biological degradation was observed after 250 days. Unfortunately, even in the bioaugmentation lane, large amounts of parent product were still reaching the effluent, and this in a very small narrow plume intersected with three injection wells. In this experiment, there was only one bioaugmentation event, and it is possible that the culture did not establish itself over the whole plume. (or no) ??

The results provide some evidence that the bioremediation approaches had any negative impact on amphibian embryos. Bacteriological screening of tissues from *Xenopus* exposed to the effluents will be direct indicators of any adverse effects not detected by the reported end-points. Additional information on the partitioning/identifying of the bacterial population in the three lanes will help assessing the biosafety of bioaugmentation compared to the biostimulation and the natural attenuation approaches.

ACKNOWLEDGEMENTS

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