I. Abstract

Waterborne illness is one of the world's most pervasive causes of morbidity and mortality, with severe health, social, environmental and economic impacts. Human faeces are often inadequately managed and contaminate community and household water supplies. Sustainable safe community and household water access is often not achieved in the developing world and adverse health impacts can result from faecal contamination of water. Metals, particularly copper and zinc, have been proposed as effective disinfectants of drinking water. This research aimed to quantify the overall disinfecting effects of a 0.2 mg/L and 2 mg/L dose of copper and zinc oxide nanoparticles, as well as the kinetics of such disinfection, on three test organisms; *Bacillus cereus* spores, *Escherichia coli* log-phase cells, and MS2 coliphage. Experiments were conducted in pH 7.3, 10 g/L (approximately 42 mM) HEPES-buffered water with and without about 10 mg/L added natural organic matter (NOM). The concentrations of culturable microorganisms were assayed at 0, 20, 60, 180, and 360 minutes. Copper and zinc oxide nanoparticles were found to be effective for same-day or overnight achievement of the World Health Organisation (W.H.O.) "protective" level of disinfection (2 $\log_{10}$ or 99% reduction) for *E. coli* in both experimental waters, and *B. cereus* spores in waters without added NOM. Measurable inactivation was not observed against MS2 coliphage for the low dose, although some inactivation (0.53 to 0.78 $\log_{10}$ reduction in 6 hours in NOM-amended and NOM-negative waters, respectively) was achieved at the high dose. Some other experimental conditions yielded inactivation, but it was not rapid or extensive enough to achieve the W.H.O. protective performance target for same-day or overnight use. Overall, the combined use of copper and zinc nanoparticles shows promise for effective point-of-use disinfection of water low in turbidity and natural organic matter.

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V. Introduction and Background

The disease burden and mortality of vulnerable individuals, particularly the poor, women, children, and rural residents, who suffer from illness as a result of drinking contaminated water, is of a great magnitude. According to the World Health Organisation in 2012, over 2.5 billion people lived without access to improved sanitation, and nearly 800 million didn't receive their water from improved sources. The term "improved" sources also doesn't necessarily imply microbial safety; many "improved" sources are still microbially contaminated in developing countries.

Approximately 1.5 million children die of diarrhoea, out of roughly 2.5 billion childhood diarrhoea cases, on an annual basis. Waterborne illness in general accounts for four per cent of all deaths and five per cent of all health loss due to disability worldwide; and diarrhoea alone is considered to be the second leading contributor to the global burden of disease (W.H.O., 2010).

Much of this disease results from inadequate provision of safe drinking water in the household. Failures in such safe water access, depending on the context, can range from the community at large, to the neighbourhood, or to the individual home. The work discussed here attempts to address the lack of safe water access at the level of the household, and potentially the neighbourhood. Although application of safe water technology can be scaled to community-level water treatment, the methodology, data, and conclusions will be presented here presuming future small-scale, household or individual usage.

This presumption of use at the household level was made based on the recognition of household water treatment systems as a valid and appropriate means of disinfecting drinking water at small scale (Sobsey 2002, Fewtrell 2005). Typical ease of use and availability, coupled with potential for low-cost high-yield efficacy implies that household water treatment systems (HWTS) can often

provide benefits in the microbial safety of water comparable to community-level treatment. In low-resource environments, HWTS can often provide more site-specific and potentially more effective coverage than poorly-implemented and managed community-level treatment systems (Clasen, 2007).

However, there are many obstacles to overcome when considering means of treating drinking water at the household level, particularly in low-resource contexts. Cost is one; granted equal efficacy, usability, and accessibility, the less expensive a method of treatment, the better. Another important consideration regarding cost is the nature of the capital required for the technology's implementation, i.e. whether it is a high initial capital investment such as for a durable good like a water filter, or a repeating, more moderate capital investment, such as a consumable good in the form of a single-use chemical disinfectant.

Durability and mobility are also issues; any method whose lack of sturdiness would lead to logistical difficulties in implementation would be of little use in resource-limited and harsh field conditions. In a related fashion, the manner in which a method is used must likewise be taken into account; any which require a high degree of education/training, or is excessively intricate would likely be limited in applicability. Finally, consumer acceptability is a key consideration. Without actual uptake and continued effective use, even the most laboratory-effective water treatments will fail to realise their full potential in the field.

Currently available methods for so-called "Point of Use Household" (POUH) water treatment fail or are deficient in one, or more of the above criteria. Free chlorine, a widely used and moderately effective disinfectant, has many undesirable qualities (e.g. chemical volatility, unpleasant odour and taste, potential carcinogenesis when combined with aqueous organic matter, ineffectiveness against

the protozoan parasite *Cryptosporidium*, etc.) that prevent it from being the ideal solution. Physical field treatment methods that are commercially available and can be utilised with water storage vessels, such as portable UV lamps, or household filters, can be acceptable to the consumer, but some have limited or uncertain efficacy against all classes of microbes (especially viruses), many pose challenges for transport because they are either too heavy or too fragile, some are of questionable durability in harsh field usage, and many have high initial cost beyond the means of low income consumers.

Chemical disinfectants such as chlorine are only moderately effective, difficult to maintain in an accessible supply chain, sometimes difficult to produce in consistent quality and concentration, cumbersome to store in liquid form, and often lead to consumer dissatisfaction due to objectionable taste, or olfactory effects. Physical filtration methods, though often durable, are typically immobile (sometimes difficult to transport due to their weight, bulk or fragility), are often ineffective against viruses and can also be expensive, requiring both a high initial capital output and periodic capital investments (Sobsey, 2010). Methods that combine two or more technologies, including the above, can mitigate or entirely overcome consumer dissatisfaction due to taste, or olfactory effects, but some fail to provide mobility/ease of portable usage, and/or are often priced out of reach for those most in need (Sobsey, 2010).

Certain antimicrobial metals have the potential to overcome one or more of these difficulties. The addition of chemically stable metals to water, in consumable amounts, would meet the criteria for durability, mobility, safe storage, and ease of use. Depending on the metal, high supply availability coupled with ease of safe storage and durability can result in a reliable supply chain and low cost. Also, depending on the metal, doses low enough to be consumable can potentially be effective and yet cause no objectionable taste, colour, and smell of treated waters. Uncharged metal

nanoparticles in particular have been considered for use as general biocides or even as POUH water treatment alternatives in the past. Uncharged silver has been repeatedly investigated as a control agent for infectious microorganisms (Qin, 2005); particularly in the context of hospital use (Atiyeh et al, 2007). Titanium dioxide has also been investigated as a drinking-water treatment both in its pure form (Ilyas and Oazi, 2010) and when doped with silver (Liga, 2011). In general however, much uncertainty and doubt exists about the health and environmental risks posed by using such metals, particularly ones such as titanium dioxide and silver that present high risks of adverse toxicological effects (Shatkin, 2008). Since the goal of this experimentation was to help identify a *consumable* disinfectant, such toxicological concerns were important to bear in mind when considering the future usability of the metal oxide nanoparticles tested here.

The research described here aimed to further characterise the potential for uncharged copper and zinc particles to be used as drinking-water disinfectants. If effective, uncharged copper and zinc, when used in combination, would meet the criteria for low cost and capital investment burdens, mobility, and simplicity of use at a minimum. Pending further work regarding the design and delivery of such a disinfectant, the criteria for stability and consumer acceptability could also be achieved. In addition, acceptable consumed concentrations for copper and zinc, depending on the form of nanoparticle used, tend to be higher than those of silver, or titanium dioxide (Shatkin, 2008), but available data are limited due to the feasibility of studying human health effects after exposure to such nanoparticles.

Uncharged copper, used alone, has been previously investigated for point of use disinfection of bacteria in water (Sudha et al, 2009). The ionic forms of copper alone, as well as copper and zinc in combination have also been preliminarily investigated as part of the larger effort of which this research was a part (Armstrong, 2011; Komandur, 2011; Malone, 2012). Furthermore, ionic copper

in combination with other metals as a biocide has been the subject of previous studies (e.g. Lin et al, 1996; Cervantes et al, 1994; Thurman and Gerba, 1989).

Previously established uses of copper as a biocide, the propensity for copper to remain in the ionised form when combined with zinc in natural waters, the use of zinc as an oral anti-diarrhoeal supplement (W.H.O., 2008) and the known propensity of zinc to selectively inactivate certain types of viruses (Sobsey, 2010) all suggest a possible use for the two elements in combination. Copper ions and zinc ions used together, particularly in combination with other methods of water treatment, have been previously studied (e.g. Abad 1994, Pyle 1992, Yahya 1990). However, the efficacy and disinfection kinetics of uncharged copper and zinc nanoparticles, used together, on a variety of microorganisms and in various chemical environments, remain relatively uncharacterised.

With the goal of eventually developing a disinfectant comprised of uncharged copper and zinc nanoparticles, an investigation regarding the effect of such metals on the survival and culturability or infectivity of three different organisms, in two different aqueous media and using two different metal nanoparticle doses was pursued. The investigation was performed using a batch disinfection method, whereby known quantities of microorganisms in test waters were simultaneously subjected to various defined disinfection conditions, and the measurable quantity of microbial survivors quantified over time. The research here, though an experimental set-up under laboratory conditions, was performed in such a manner as to approximate typical household use conditions as much as possible.

VI. Literature Review and Experimental Context

A basis exists for the belief that copper and zinc oxide nanoparticles may perform as adequate point-of-use-household (POUH) drinking-water treatments. Copper ions alone as well as copper ions in combination with other metals and/or chlorine in particular have been studied as biocides and as disinfectants of drinking water, though not to the extent of silver ions. Metal oxide nanoparticles in general have also been studied as biocides, potentially toxic agents, and disinfectants of drinking water. Most relevant to this study are studies of titanium dioxide nanoparticles, due to chemical similarity to copper and zinc oxide nanoparticles (Badireddy, 2011). Relevant studies of the biocidal and toxicological effects of copper and zinc oxide nanoparticles in particular are also presented below. Though such studies frequently do not focus on drinking-water disinfection, nor on disinfection or inactivation kinetics, they still remain useful as a theoretical basis for any antimicrobial activities that may be expected or observed.

Copper Ions (Copper Salts) Studied Alone

Copper sulphate in salt form has been investigated as early as the beginning of the 20th century, as an algaecide (Moore et al, 1904). Treatment with copper sulphate was further investigated as a complement to chlorination for the purpose of controlling algal growth in commercial water facilities such as swimming pools. Fitzgerald et al (1963) investigated copper sulphate, dosed to deliver 2 mg/L of aqueous cupric ion, as an antagonist to *Microcystis aeruginosa* and *Chlorella pyrenoidosa*. Copper sulphate at these concentrations was observed to fully inhibit algal growth (no proliferation observed) in liquid algal culture when compared to a copper-negative control. Zevenhuizen et al (1979) studied the inhibition of bacterial growth by copper ions. Their study found that a wide range of cupric ion concentrations, between 10^{-8} and 10^{-6} M (0.006 to 0.00006 mg/L), in inorganic salt-glycerol solutions prevented the culturability of *Klebsiella aerogenes*, when plated on non-selective solid agar medium. Inactivation of between 2 and 3 $\log_{10}$ reductions was

observed after 4 hours. Zevenhuizen et al did not examine whether or not a similar effect would be exerted in an aqueous medium.

Domek et al (1984) studied similarly low levels of cupric ions. Their study observed injury of coliform populations caused by levels of free cupric ion between 0.007 and 0.54 milligrams per litre. A variety of natural waters sampled from mountain streams were used as the experimental medium. Laboratory experiments with these natural waters (pH varying between 7 and 8) used 0.25 and 0.5 milligrams of cupric ion per litre as the test doses. The time taken to achieve 1 $\log_{10}$ reduction of coliform bacteria was reported for both doses; the 0.25 mg/L dose required 6 days of contact time, while the 0.5 mg/L dose required 2 days of contact time. Domek et al (1987) further performed metabolic studies. Injuries in the respiratory chain of tested *Escherichia coli* were deduced, based on reductions in oxygen demand of bacterial colonies at room temperature. Such deduced injuries were thought to explain observed reductions of > 2 $\log_{10}$ when dosed with 0.117 mg/L cupric ion for 7 days.

Thurman and Gerba (1989) posited that any inactivation of microbes by cupric ion activity may proceed via the ligand-induced alteration of tertiary or quaternary structure of essential proteins in the microorganism. Raman et al (2009) in their study found *in vitro* evidence to support this claim. Synthetically-produced Shiff Bases (protein structures that are identical to those found on the surfaces of many microorganisms) were analysed after exposure to cupric ion using spectrophotometric analytical techniques, and were found to form square-planar complexes after exposure, but not before. Such complexes were further found to form intercalating DNA lesions, using electronic absorption spectroscopy, and differential pulse voltammetry. Though their study was not performed on live microorganisms, it is possible that such effects occur *in vivo* as well.

Cupric ion-based complexes in water may participate in disinfection indirectly as well. Kuwahara et al (1986), Vasudevachari (1982), and Samuni (1983) have all observed the production of hydroxyl radical species by copper complexes in water; these radical species were theorised to induce observed inactivation of viruses. Samuni et al (1983) studied concentrations of approximately 0.13 mg/L, cupric ion, which induced the production of hydroxyl radicals when placed in phosphate-buffered pH-neutral laboratory water. Experimental waters were spiked with culturable phage Lambda, as well as phages T2-T7. Surviving phages were plated on *E. coli*-seeded tryptone agar plates, and plaque-forming units quantified. Up to 2.5 $\log_{10}$ reductions were observed after 30 minutes; Chick-Watson disinfection kinetics were observed. Kuwahara et al (1986) observed approximately 2 $\log_{10}$ reductions (as measured by reduction in plaque-forming units, or PFUs, on *E. coli*-seeded solid agar medium) over a contact time of 180 minutes when bacteriophage phi-X174 am3 was exposed to cupric ion at concentrations varying between 1.3 and 3.2 mg/L in borate-buffered waters of pH 7.6 at an unspecified temperature. Sterrit and Lester (1980) found that cupric ions disrupt synthetic and naturally-obtained enzymes by binding to electronegative amino acid functional groups in their protein structure. Manzl et al (2004) found that cupric ions exhibit a peroxidising effect on lipids.

Noyce et al. (2006) found that cupric ions leached from copper-cast alloy coupons as compared to a stainless-steel control coupon. Further experimentation on *E. coli* 0157:H7 was performed. *E. coli* was used to inoculate extracted beef juice, and subsequently was exposed to metal coupons whose percent composition of copper varied from 0 to 95% for up to 6 hours of contact time at two different temperatures (22 and 4 degrees centigrade). At 22 degrees Centigrade after 6 hours of contact time, no CFUs were found from samples from all copper-containing coupons when compared to a copper-negative control. At 4 degrees centigrade, after the same contact time, only alloys of 94% and 95% copper yielded statistically significant (i.e. non-zero) reduction (measured

at 1.5 and 5 $\log_{10}$ reductions, respectively). No statistically-significant reductions were found in samples taken from inoculum exposed to copper-negative controls.

Gadd and Griffiths (1977) as well as Cervantes et al (1994) established that aqueous cupric ion promoted selection of genetically distinct microorganisms; it is theorised that an effect of natural selection induced the presence of traits conferring resistance to copper toxicity. Mintz et al (2005), in a comprehensive literature review that considered papers published on POU(H) treatment and storage of water containing enteric indicator organisms, established copper (II) sulphate as a potential POUH drinking water treatment technology, positing efficacy at doses above the consumable level, though kinetics and quantification were not reported in detail.

Lehtola et al (2004) studied biofilm formation and proliferation in water distribution systems (roughly pH neutral water at roughly room temperatures) comprised of copper pipes, as compared with polyethylene (PE) pipes as a control. The formation of biofilms and their proliferation were measured by heterotrophic plate counts and ATP concentrations (per unit volume) in sampled biofilm material. Observations were made over 308 days with samples being taken on a daily basis. Formation of biofilms were not found to be significantly different at the end of the experiment. However, in the first 200 days of the experiment, biofilm formation in copper pipes was statistically significantly reduced as compared to pipes made with polyethylene. Copper pipes had 20% the ATP of PE pipes, on average, and reductions of approximately 75% in growth on heterotrophic plates were found prior to 200 days. In copper pipes, aqueous cupric ion concentrations varied widely, but never exceeded 0.7 mg/L.

Van der Kooij et al (2005) performed a similar study. Theirs compared biofilm formation of *Legionella* in model warm-water systems comprised of copper pipes, stainless steel pipes, and PE

pipes when grown in tap water between 25 and 35 degrees centigrade and subsequently exposed to waters of 70 degrees centigrade twice weekly. Median values of ATP concentration for copper pipes were 2.1 ng/L, as compared with 2.5 ng/L for stainless-steel pipes and 4.5 ng/L for PE (control) pipes. This findings were mirrored when using biofilm formation as a metric; values for copper and stainless-steel pipes were similar (630 pg/cm^2) and both were significantly less than PE (control) pipes (1870 pg/cm^2). *Legionella* proliferation was measured at 1500 CFU/L in copper pipes, and approximately 4300 CFU/L for both stainless-steel and PE (control) pipes. Copper pipes were found to significantly impair *Legionella* proliferation when compared with stainless-steel, though stainless-steel performed similarly as an inhibitor of biofilm formation and proliferation.

Dwidjosiswojo et al (2011) further found that doses of copper ions of approximately 0.65 mg/L exhibited 6 $\log_{10}$ reductions of *Pseudomonas aeuruginosa* culturability (as measured by CFUs on solid-agar plates) after 24 hours of exposure at 20 degrees centigrade in roughly pH-neutral tap water obtained from domestic plumbing systems. Total cell numbers determined "microscopically" did not decrease however, and the amount of intact 16S ribosomal RNA (as determined by fluorescent *in situ* hybridisation) remained roughly equal before and after exposure. Complete restoration of culturability was found after 14 days when copper-exposed bacterial samples were dosed with a chelating agent (diethyldithiocarbamate, or DDTC) found to remove cupric ion from biological ligand-based complexes.

Copper Ions (Copper Salts) Studied in Combination with Other Metals (Metal Salts)

Kim et al (2004) determined that ceramic filters infused with a metallic disinfectant comprised of copper, silver, and other metal ions in unknown proportion was capable of achieving a 2 $\log_{10}$ reduction in culturable microorganisms in just 15 minutes. Details of the filter, due to its status as a protected trade secret, were not provided in the paper. A trademarked "EEKO-BALL" stock solution

of unspecified composition but containing 0.05 mg/L silver ion and 0.05 mg/L copper ion, of pH 7.3 was inoculated with *E. coli* at 25 degrees centigrade, and found to be four times as effective at disinfection (contact time required to reach 2 $\log_{10}$ reductions of 16 minutes, as opposed to 58 minutes) when compared with the same concentrations of silver and copper ions present in a solution of equal pH and temperature, but without EEKO-BALL's secret ingredients.

Stouts et al (2003) performed a comprehensive metal-analysis of data reported in a literature review of long-term (>365 day) hospital studies aimed at characterising the efficacy of copper-silver ionisation systems at controlling *Legionella pneumophilia*. Using measures of oxygen demand and ATP metabolism as surrogates for biofilm activity, their study found that hospital warm water systems that utilised copper-silver ionisation at a wide range of concentrations exhibited disinfection of *Legionella* to a degree that was statistically significantly different from hospital warm-water systems. The measure used was reports of samples taken from such systems that tested positive for *Legionella*. After the installation of copper-silver ionisation systems, half of all hospitals reported no positive samples, and an additional 44% reported a proportion of positive samples less than 3 in 10, as compared to all hospitals reporting approximately 90% positive samples prior to system implementation.

Blanc et al (2005) and Cachafeiro et al (2007) also performed critical evaluations of copper-silver ionisation systems used in hospital settings as disinfectants, most particularly for *Legionella pneumophilia*. Blanc et al reported that no significant difference was found in disinfection of *Legionella* between copper-silver ionisation systems as compared to ozonation systems when the water temperature was either 50 degrees centigrade or 65 degrees centigrade. Cachafeiro et al selected ten studies published between January 1997 and January 2007 for a systematic review and meta-analysis. For ion levels that ranged from 0.1 mg/L to "within internationally recommended

levels" of 2 mg/L for copper, Cachafeiro found that copper-silver ionisation was an "effective" method of disinfection using the reduction in the percentage of samples that tested positive for *Legionella* as the criterion for judgement. Statistical significance was calculated in all cases using a two-sample proportion (z) test for significance, disinfection kinetics (if studied) were not reported.

Titanium Dioxide Nanoparticles

Reviews of the safety and efficacy of various metal nanoparticles as biocides have been performed, though few in an experimental context relevant to drinking water. Most relevant to this experimentation are studies performed on titanium dioxide nanoparticles (usually doped with silver or nitrogen). Yang-Hwei et al (2007) performed a comprehensive literature review of titanium dioxide nanoparticle-induced photocatalysis in aqueous solutions. Photo-catalysis is theorised to be the "active ingredient" of TiO_2 nanoparticle-based disinfectants. Such nanoparticles (un-doped) when embedded in the surface of a model orthopaedic implant in combination with UV light were found to induce over 3 $\log_{10}$ reductions in 50 minutes of culturable *E. coli, P. aeruginosa, S. aureus, E. hirae*, and *B. fragilis* grown on a model orthopaedic implant at room temperature; such populations were also exposed to UV light without titanium dioxide nanoparticles and no significant disinfection was observed in the time period studied. Brunet et al (2008) found that un-doped titanium dioxide nanoparticles in pure water (at a concentration of approximately 100 mg/L), produced hydroxyl radicals when exposed to light; such production was theorised to induce bacterial inactivation (75% of *E. coli* exposed were inactivated after 6 hours). The production of hydroxyl radicals was not found when bacteria were exposed to identical nanoparticles at the same concentration in dark environments.

Liga et al (2010) observed the efficacy of silver-doped titanium dioxide nanoparticles for disinfection of MS2 coliphage viruses in dark and in light. For dark experiments, silver-doped TiO_2

nanoparticles were compared with un-doped nanoparticles and a metal-negative control. Sampling was taken after 10 minutes of agitated contact time at room temperature; subsequently, the samples were kept at 4 degrees Centigrade before a final enumeration. Un-doped nanoparticles achieved approximately 20% inactivation after 10 minutes, doped nanoparticles achieved approximately 80% inactivation after the same time period. This difference was attributed to leached silver ion. The dark mechanism of action of titanium dioxide was theorised to be aggregate formation with virus particles, lending credence to the belief that copper and zinc oxide nanoparticles may perform in a similar manner.

The same experiment was repeated in a photo-catalytic context, with exposure to light at 350 nm at 2.5 milliwatts per square centimetre of intensity. The production of hydroxyl radicals was observed. Both doped and un-doped nanoparticles yielded significant disinfection; un-doped nanoparticles yielded approximately 2 $\log_{10}$ reductions in PFUs after only two minutes, while doped nanoparticles achieved approximately 6 $\log_{10}$ reductions in PFUs in a similar time period. Photo-catalytic production of hydroxyl radicals is theorised to account for the difference in virus reduction results between dark and light experimentation.

Copper and/or Zinc Oxide Nanoparticles

Copper and zinc oxide nanoparticles have been specifically investigated as toxicants and biocides. Aruoja et al (2008) experimented with copper and zinc oxide nanoparticles, in comparison to the "bulk" (lumps visible to the naked eye) form of the metal oxides. The alga *Pseudokirchneriella subcapitata* was studied for ability to grow when dosed with nanoparticle and bulk ZnO, CuO, and TiO_2 at doses between 0.04 and 1 mg/L in aqueous solution. The concentration required to achieve 50% growth inhibition of the organisms (EC50 concentration) was observed. All populations exhibited EC50 levels within this dose range; algal proliferation was measured in standard aqueous

medium, and the dose at which 50% of algal growth was inhibited was noted. More relevant to this study, relative effects of the nano- and bulk form of copper and zinc oxide were determined. Nanoparticle copper oxide produced approximately 141 times as much "bioavailable" (free) copper as bulk copper oxide. The same was not the case for zinc oxide; nanoparticle zinc oxide was found to produce bioavailable zinc at similar levels as bulk zinc oxide. Zinc oxide nanoparticles were, however, found to be *more* toxic to microalgae than copper oxide nanoparticles; both were found to exceed titanium dioxide in toxicity. Specific kinetic quantification of viability reductions in the microalgal populations was not performed in these studies.

Kasemets et al (2009) reported similar findings regarding differential performance between copper and zinc oxide in the nanoparticle form. "The effect of metal oxide nanoparticles, their bulk forms, and respective ionic forms were compared". All biocides were dosed in the growth medium of the yeast Saccharomyces *cerevisiae* (malt extract medium of 20 g/L density at 30 degrees centigrade). Toxicity findings, as measured by inhibition of *S. cerevisiae* were similar to those observed by Aruoja et al. for algae. More relevant to this study, the finding that both nano- and bulk ZnO were similar in toxicity was replicated. After 8 hours of contact time, the EC50 of both were between 121 and 134 mg/L. After 24 hours of contact time, the EC50 of both were between 131 and 158 mg/L. Nano-CuO was found to be approximately 60 times more toxic than the bulk form. After 8 hours of contact time, EC50s were 20.7 and 1297 mg/L for the nano and bulk form, respectively. After 24 hours of contact time, EC50s were 13.4 and 873 mg/L, respectively. By using cupric ion dosing as a control for ionic activity, the authors concluded that the availability of cupric ion accounted for only "50% of the toxicity of both nano and bulk CuO". Information for zinc ion dosing and the statistical effects of bioavailable zinc ion in disinfection experiments with nano and bulk ZnO was not reported.

Heinlaan et al (2007) examined the effect of these metal oxide nanoparticles on *Vibrio fischeri* and several species of crustaceans *in vivo*, in a 2% NaCl aqueous solution (near-neutral pH) intended to mimic saltwater chemical environments. CFUs for *V. fischeri* were quantified on standard solid agar medium. Examinations of the effect of these metal oxide nanoparticles mirrored the findings of Kasemets et al and Aruoja et al; zinc ions exhibited similar toxicity to zinc oxide nanoparticles whereas nanoparticle CuO was much more toxic than bulk Cu. Contrary to the findings of Kasemets however, nearly 100% of the toxic effect of CuO was found to be due to cupric ion release, as compared with an ion-only control sample. Brayner et al (2006) examined ZnO nanoparticles (delivered in diethylene glycol, or DEG, medium) alone as disinfectants of *E. coli*. Nanoparticle dosing was performed on (and CFUs were quantified on) Luria-Bertani medium (solid agar plates). Transmission electron microscopy (TEM) was used to study metal-stressed bacterial populations. "The results confirmed that *E. coli* cells after contact with DEG and ZnO were damaged showing a Gram-negative triple membrane disorganisation".

Inactivation data or kinetics were not the specific focus; rather, the focus of the study was to determine the potential mechanism for ZnO inactivation of microorganisms. Brayner et al (2006) found that perforations were made in the cellular membrane of ZnO-stressed *E. coli*, leading to internalisation of the nanoparticles and subsequent inactivation. Binding to lipids and proteins increased drastically in *E. coli* O157:H7 that were exposed to nanoparticle zinc oxide at doses between 10^{-2} and 10^{-4} M (6.35 to 635 mg/L) and inactivation varied between 100% at the higher dose and 85% at the lower dose within 6 hours.

Luna-delRisco et al (2010) performed a study on the effect of copper and zinc oxide nanoparticles in combination on the production of biogas (taken to be an experimental surrogate for microbial activity) in anaerobic digestors. Temperature varied with microbial activity in the digestors, as did

ambient atmospheric pressure, nutrient availability, etc. Activity in digestors containing bulk- and nano-sized CuO and ZnO was compared with biogas production in a metal-negative control digestor. EC50 doses for nano-CuO were estimated to be 10.7 mg/L, and for nano-ZnO were estimated to be 57.4 mg/L, leading to the conclusion that both metal oxide nanoparticles in combination significantly retarded microbial activity in such environments.

Summary of Reviewed Literature

1) Copper salts, when studied alone, are found to be effective disinfections of algae, bacterial colonies, biofilms and bacteriophages.
2) Cupric ion has been shown to exhibit ligand-based complexation reactions with synthetic proteins that are structurally similar, or identical, to those found on the surfaces of microorganisms
3) Cupric ion has been demonstrated to injure the metabolic systems of coliform populations
4) Populations stressed with cupric ion have been shown to evolve to become genetically distinct from non-stressed populations over time
5) Cupric-ion-based complexes in aqueous environments have been shown to produce hydroxyl radical species, a known microbial inactivator.
6) Cupric ions have been found to disrupt synthetic and naturally-obtained enzyme activity, as well as to exhibit a peroxidising effect on lipid structures
7) Cupric ions have been theorised as the explanation for the observed inhibition of biofilm growth and proliferation on copper pipes, as compared with polyethylene or stainless-steel controls
8) One study has found that organisms exposed to cupric ion have the capacity to recuperate after 14 days once exposure has been removed. Furthermore, exposure to cupric ion

prevented culturability but did not decrease measurable cell counts or the amount of intact 16S ribosomal RNA.

9) Copper-silver ionisation systems have been broadly demonstrated to be effective at controlling growth and proliferation of *Legionella pneumophilia*, particularly at higher temperatures.

10) Titanium dioxide metal nanoparticles are effective disinfectants in aqueous media; their efficacy is amplified by doping with silver or nitrogen. Their efficacy is theorised to be due to photo-catalytic production of hydroxyl radicals under near-UV or UV light; experimentation in the dark produces only a slight effect, most likely due to aggregation and complexation reactions with microorganisms.

11) Nanoparticle zinc oxide and bulk zinc oxide perform similarly against a variety of microorganisms in near-pH neutral waters; both are moderately effective disinfectants in isolation. The degree to which such disinfection is due to the production of zinc ions in aqueous media is uncharacterised.

12) Nanoparticle copper oxide exhibited significantly higher disinfection than (exhibited an effect amplified by anywhere from 60-141 times more than) bulk copper oxide. Both are effective as disinfectants in isolation. Approximately 50% of the disinfecting effect of copper oxide, whether in the nanoparticle or the bulk form, is attributed to the production of cupric ion in aqueous media.

13) Nanoparticle zinc oxide has been found to bind to and perforate the cellular membranes of *E. coli*, leading to their subsequent internalisation. This is theorised as the mechanism behind observed disinfection of *E. coli* by nanoparticle zinc oxide.

14) Copper and zinc oxide nanoparticles, studied together, were found to significantly retard biogas formation (a surrogate for microbial activity) in anaerobic digestors as compared with a metal-negative control digestor.

From the reviewed literature above, a basis exists for the thesis that copper and zinc oxide nanoparticles, when dosed in combination and used against bacteria, viruses, and protozoan (oo)cyst surrogates, may be effective disinfectants. Though inference into the mechanism by which such potential disinfection may occur is not the focus of this study, the results presented herein may contribute to future attempts to elucidate the means by which such disinfectants achieve inactivation of test microorganisms.

Information on the specific kinetics of inactivation, as well as the use of "safe" (consumable) doses of these disinfectants in water intended for human consumption is scarce, as is information on their effect in waters containing natural organic matter (NOM) and the effect, if any, such NOM may have on the disinfection effects observed. An evaluation of the ability of copper and zinc oxide nanoparticles to meet the W.H.O. "protective" performance targets of 2 $\log_{10}$ reductions of culturable bacteria and protozoan (oo)cyst surrogates and 3 $\log_{10}$ reductions of enteric virus surrogates has not been found in the literature. Likewise, a specific evaluation on the ability of copper and zinc oxide nanoparticles to meet acceptable disinfection (by any standard) of bacterial, enteric virus, and protozoan (oo)cyst surrogates in a context consistent with same-day or overnight consumable disinfection has not been found. The experimentation discussed in this technical report aims to help elucidate such matters.

VII. Objectives and Experimental Approach

Objectives

This experimentation aimed to test the hypothesis that a metal additive comprised of uncharged copper and zinc nanoparticles or a chemically related form of these metals could be a feasible alternative disinfection treatment method for drinking water at the point of use (household) level. Feasibility is defined by the following criteria:

1. The reaching of an adequate magnitude of disinfection performance by a dose of the metal additive suitable for safe daily consumption
2. The achievement of this performance against a representative range of microorganisms
3. The achievement of this performance within a time period consistent with same-day or overnight use.

Therefore if the metal additive tested here were to not achieve an adequate level of disinfection performance across all classes of microorganisms tested within approximately 12-18 hours at the doses employed, it would be considered inadequate for application as a consumable point-of-use (household) drinking water treatment.

Experimental Approach

Metal Oxide Nanoparticles

The focus of this research was to evaluate the use of uncharged nanoparticles of copper and zinc to disinfect drinking water. Any biocidal properties have been theorised to reside in the surface chemistry (reactivity) of these metals, and their propensity to release metal ions in aqueous solution (Singer, 2010). In order to maximise the amount of potential surface chemistry therefore, the nanoparticle forms of these metals were chosen. Because smaller size corresponds to a larger surface-area-to-volume ratio, the nanoparticle forms of these metals present larger total surface

area per unit mass than an equivalent mass of uncharged particles in the "bulk" (macroscopically-sized) form.

For this reason however, nanoparticles tend to be very highly reactive (Shatkin, 2008). For the purpose of consumable drinking-water disinfection, a certain amount of stability is desirable. Pure uncharged nanoparticle copper and zinc, though available, have very poor shelf life and storage capacity. Upon exposure to ambient air, they very readily form mono- or occasionally poly-oxygenated species, and react with water particles, environmental contaminants, and other heterogeneous airborne compounds to create complexes of unknown size, chemical activity, and prevalence (Badireddy, 2011).

For these experiments, the nanoparticle forms of copper and zinc were stabilised prior to use. Due to the likely high level of experimental precaution that would have been required in order to prevent the formation of mono-oxygenated species (oxides) of these metals upon exposure to ambient air, and due to the desire to use forms of the metals that would have relatively simple usage instructions in low-resource environments, the oxide forms of these metals were chosen. In this research, nanoparticles of cupric oxide (CuO) and zinc oxide (ZnO) were used, ranging in size between 25 and 45 nanometres. One particle of metal oxide was considered, for the purposes of this experimentation, to be one particle of "deliverable metal". The particles were stored in separate colloidal solutions, using an aqueous solvent matrix without other nanoparticles, without compounds that would obscure, bind with, or induce precipitation in the desired nanoparticles, under conditions of darkness, and were refrigerated at a temperature of 4 degrees Centigrade.

Dose

The World Health Organisation, the US Environmental Protection Agency and the United States Food and Drug Administration have published recommended levels of copper and zinc for drinking water (W.H.O., 2004; W.H.O., 2007; F.D.A., 1994; E.P.A., 2002). Copper and zinc have different tolerable maximum levels. The W.H.O and the F.D.A both recommend a maximum limit of 2 milligrams of copper per person per day, or 0.05 milligrams of copper per kilogram of body weight per person per day. A maximum concentration of 2 milligrams of copper per litre of water is also recommended by both parties. The E.P.A., based largely on consideration of at-risk subpopulations, such as those with Wilson's disease, recommends a maximum of 1.3 mg/L of copper, though this level is non-enforceable.

For zinc, the recommended limits by both the F.D.A. and the W.H.O. are the lower of 15 milligrams per adult per day or 0.375 milligrams per kilogram of body weight per individual per day. However, the recommended maximum concentration in drinking water is 2 milligrams of zinc per litre of water for the F.D.A. and W.H.O., and 5 milligrams of zinc per litre for the E.P.A., based on aesthetic factors such as smell and taste. 2 milligrams per litre, the lower of the two limits, is designated as the maximum concentration in this research (W.H.O. 2007). The recommended maximum aqueous concentrations for both copper and zinc for this research were therefore both equal, and are denoted henceforth as the "100%" dose.

In addition to the 100% dose, a tenfold reduction of metal additive was also used in experimentation, corresponding to 0.2 milligrams per litre, to facilitate future analysis of dose-response data. In addition, during preliminary experimentation (see for example Armstrong, 2011; Malone, 2012), a dose corresponding to 30% of the maximum aqueous concentration, or 0.6 milligrams per litre of metal additive was used. However, initial evaluation performed during the

course of experimentation indicated no statistically significant difference between responses induced by the middle dose, and responses induced by the low dose. The use of the 0.6 milligrams per litre dose was therefore discontinued in order to conserve laboratory resources. Finally, a negative control dose, corresponding to no addition of metal additive, was evaluated throughout the course of the experimentation. In every other respect, samples containing the control dose were treated identically to samples containing the 10%, or the 100% metal nanoparticle dose in order to facilitate scientifically valid and statistically rigorous comparison.

Test Microorganisms and Added Concentration to Test Water

Three test microorganisms were used in these experiments: *Bacillus cereus* spores, log-phase *Escherichia coli* "B" strain, and MS2 bacteriophage. *Bacillus cereus* spores were used as an experimental surrogate for (oo)cysts of protozoan parasites and spores of other drinking water pathogens that are considered generally resistant to disinfection, such as *Cryptosporidium* oocysts. *Escherichia coli* vegetative cells were used as experimental surrogates for Gram-negative intestinal bacterial pathogens such as those from the genus *Salmonella, Escherichia, Shigella, Campylobacter* and *Vibrio.* MS2 phage virions were used as experimental surrogates for enteric viruses, such as enteroviruses, Hepatitis A and E viruses, noroviruses or astroviruses.

B. cereus was used in its spore form; stock spore suspensions were ordered from the American Type Culture Collection (ATCC), and were standardised at a concentration of 10^6 spores per 0.1 millilitre. MS2 bacteriophage virions were used in the form of a thawed stock suspension prepared initially as the harvested top agar from double layer plaque assay plates that were centrifuged at 5000 g for 15 minutes at room temperature to remove agar and cell debris. (for more details see Armstrong, 2011); MS2 stocks grown in *E. coli* F_{amp} host were kept frozen at -81 degrees centigrade and thawed immediately prior to experimentation.

Organisms were added to test water prior to disinfection experimentation in doses calibrated to enable a potentially measurable 4 $\log_{10}$ (99.99%) reduction. Lower detection limits for organism assays were taken as 5 culturable organisms per 100 microlitres of plated sample, which corresponded to an initial "target" overall concentration of $5*10^6$ (5 million) culturable organisms per litre. This concentration was taken as the minimum approximate target dose of organisms in test water during this research. Since several serial 10-fold dilutions were prepared and at least three were assayed by default, a wide range of initial and subsequent concentrations of culturable organisms per litre were capable of being assayed. In a similar manner, the upper detection limit of approximately 500 culturable organisms per 100 microlitres of plated sample corresponded to an initial "target" overall concentration of $5*10^8$ (500 million) culturable organisms per litre. Initial dosing of test waters therefore ranged between these two values (5 million and 500 million culturable organisms per litre).

Test Waters

The focus of this research requires consideration of end-use feasibility. To this end, an important factor in this experimentation was the consideration of the effects (if any) of dissolved aquatic organic matter on the potential efficacy of copper and zinc nanoparticles as disinfectants of drinking water. Two types of test waters were therefore used in this research.

The first was chemically-defined, autoclaved, deionised water, buffered to a pH near 7.5, and containing no other added compounds. The buffer used, the "Good buffer" 4-(hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), is a zwitterionic buffer with a pKa of 7.55 (Good et al, 1966). HEPES was used at a concentration of 10 grams per litre (0.042 M) in order to prevent complexation reactions with the metal additives. With buffers such as phosphate and carbonate,

there was reason to suspect that complexation of the metallic additive with the negative ions of such buffers would decrease "bioactive" and "bioavailable" chemical forms in situ (Singer, 2010).

With HEPES, photo-toxicity has been observed in buffered solutions (Singer, 2010; Coronell, 2012). Specifically, generation of peroxide species upon exposure to light had a potential to confound the effects (if any) of the copper and zinc additives. In order to prevent such photo-toxic effects from being observed, all solutions buffered with HEPES were kept in conditions of darkness, save for brief periods of time corresponding to sampling. Control vessels, also buffered with HEPES, were furthermore monitored for any consistent decrease in microorganism concentration over time, an effect which would have manifested in the case of photo-toxicity.

The second test water was identical to the first, but with the addition of 10 milligrams per litre of dehydrated organic solids originally collected from waters of Catfish Lake, Croatan National Park, NC. The dehydrated solids were suspended within an additional 10 millilitres of Catfish Lake water per litre of buffered water solution (1% V/V). Water from Catfish Lake was gravimetrically measured, and determined to have an overall concentration of approximately 1 gram per litre of total dissolved solids (TDS). The precise composition of the TDS is unclear; total organic carbon (TOC) analysis, a measure not including inorganic carbon salts, yielded a result of 10.5 milligrams per litre in the original stock solution, implying that approximately 1% of the TDS present in the water added to the test solution was comprised of organic carbon.

In addition, conductivity analysis was performed, yielding a result of 64 microsiemens per centimetre. This value corresponds to that of a solution of water with roughly 25 milligrams of dissolved sodium chloride. From conductivity analysis, the overall activity of ionic compounds in the water can be inferred; the value implies that the stock solution of Catfish Lake water had a very

low concentration of dissolved ions. The test waters used in experimentation, without the presence of HEPES buffer (due to its potentially confounding effect as an organic compound), when amended with Catfish Lake rehydrated solids, were found by direct measurement to have a TOC concentration of approximately 10. mg/L (a measurement accurate to two significant figures).

Time Points and Sampling Methods

In order to approximate typical daytime use of stored water in low-resource settings, an overall time period of six hours (360 minutes) was considered suitable for the experimentation. In this research, sampling occurred periodically throughout the course of the experiment, in order to facilitate subsequent analysis based on possible exponential disinfection kinetics. Samples were taken at 0, 20, 60, 180, and 360 minutes, or 0, 0.33, 1, 3, and 6 hours. Intervals between time points were chosen to facilitate semi-log (i.e. log-linear) regression analysis.

In order to ensure statistical validity, sampled volumes must be representative but small compared with the overall volume of test water. In this experimentation, sample volumes were capped at 0.5% of the overall test volume of water. In the case of a 100 millilitre volume of test water, this would imply a sample volume of 500 microliters (0.5 millilitres). Five samples were taken in total per experiment; this corresponds to an overall removal of 2.5% of the test volume.

At each sampling time, the sample volume was immediately diluted 10-fold in Dulbecco's Phosphate-Buffered Saline (DPBS). For example, a sample volume of 500 microlitres would have been placed in 4.5 millilitres of DPBS. The volume of DPBS corresponding to the 10^{-1} dilution also had an amount of disodium EDTA present that was intended to ensure a final EDTA concentration of $5*10^{-4}$ M in the dilution tube.

Disodium EDTA was added as a chemical "neutraliser" in order to ensure that any bioactive form of the metal additive present in the sample was chemically bound, and thereby rendered unavailable to react with the test microbes. Preliminary experimentation using copper and zinc metal ions (Armstrong, 2011; Komandur, 2011) indicated that disodium EDTA was a suitable chemical neutraliser at the concentration used, and one that did not itself exhibit statistically significant biocidal properties under these experimental conditions. Upon this initial sampling, subsequent dilutions were made in ten-fold series in DPBS.

Bacterial and Viral Assay Techniques

B. cereus and *E. coli* were assayed on an agar medium of custom design via the spread-plate method. The medium, dubbed "Awesome Agar" is comprised of standard Difco Trypticase Soy Agar (TSA) with the addition of 10 grams of lactose per litre of desired agar solution, and 5 milligrams of Neutral Red (Toluylene Red) per litre of desired agar solution, the latter a chemical commonly used in histology. *B. cereus*, unlike *E. coli*, does not metabolically ferment lactose, a property that was used as a means of differentiating colonies of *E. coli* and of *B. cereus*, upon plating. Because the by-products of metabolic fermentation of lactose are acidic, *E. coli* bacterial colonies producing such by-products are therefore stained pink upon assay in medium containing Neutral Red. *B. cereus* colonies not producing such lactose fermentation by-products remain their original colour (usually white).

In order to evaluate the assay efficacy of Awesome Agar, preliminary experimentation compared bacterial enumeration (by spread plating) of samples taken from water contaminated at various concentrations with either or both *E. coli* and *B. cereus*. Samples were plated on standard Difco MacConkey agar (for selecting Gram-negative *E. coli* at the expense of *B. cereus*), Awesome Agar, and standard Difco TSA (a generalised growth medium that supports the growth of both *E. coli* and

B. cereus). Ideal results for water samples contaminated with only *E. coli* would indicate growth on all three media in similar amounts. Ideal results for water samples contaminated with only *B. cereus* would indicate similar amounts of growth on Awesome Agar and standard Difco TSA, with no growth shown on MacConkey Agar. Ideal results for water contaminated with both organisms would indicate similar growth between Awesome Agar and standard Difco TSA plates. Plating of such samples on MacConkey agar was not necessary for this analysis and therefore not performed.

Results were exactly as expected for most bacteria concentrations tested; *B. cereus* colonies were distinguishable from *E. coli* colonies on Awesome Agar, and Awesome Agar did not yield significantly different counts of either organism from their standard agar medium (all p-values >0.05). These findings were valid for concentrations of culturable organisms per millilitre of water that were below $5*10^8$, corresponding to a plate count of 500 colony-forming units (CFUs). Above this concentration, growth of *B. cereus* was no longer distinguishable from growth of *E. coli* due to indistinguishable blending of stained and unstained colonies on the agar plates (i.e. the colonies were too numerous to count). This value of $5*10^8$ CFUs/mL was therefore considered an absolute maximum or ceiling value for these experiments and was not exceeded during the experimentation. To assay for MS2 bacteriophage, the standard EPA Method 1602, "Male-specific (F+) and Somatic Coliphage in Water by Single Agar Layer (SAL) Procedure", was used with no significant modifications. The host bacterium was *E. coli* F_{amp}.

Disinfectant Concentration Measurement

In order to measure the concentration of copper and zinc nanoparticle disinfectant at 0 minutes, and at 360 minutes, Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) was used. ICP-MS is a preferred analytical technique due to its high resolution, ranging to below one part per trillion (one part in 10^{12}) for metals such as copper and zinc. It is based on a type of plasma produced via

electromagnetic induction and used to selectively ionise target metals for the purpose of mass spectrometry. Though potentially usable to differentiate between speciation of metals, in this case ICP-MS analysis was generalised sufficiently to detect all forms of copper and zinc not bound to microorganisms in solution or found in complexes/aggregates.

In order to analyse the effect, if any, of the presence of microorganisms on the concentration of disinfectant, samples taken from organism-positive test waters were compared with those simultaneously taken from identically-prepared organism-negative test waters. These samples were in turn standardised against a sample taken from the control (metal-free, organism-positive) observation and against a sample of "pure" water prior to analysis. In this fashion, the effect over time of disinfectant concentration was obtained, and the effect of the presence of organisms on the concentration of both copper and zinc aqueous species was inferred, though data found were of insufficiently high *n* to conduct statistically rigorous hypothesis testing.

VIII. Experimental Plan

Independent Variables: Metal Doses, Organisms and Test Waters

This experiment had three independent variables overall, the sequential manipulation of which yielded eighteen total experimental sets. The first independent variable was the metal additive dose. Dosage of the metal additive varied between three possible values: nil (as a control), 0.2 milligrams per litre, and 2 milligrams per litre each of copper and zinc oxide nanoparticles. The total amount of metal added therefore varied between nil (as a control), 0.4 milligrams per litre (0.2 milligrams each of copper oxide and zinc oxide), and 4 milligrams per litre (2 milligrams each of copper oxide and zinc oxide). Throughout the course of this experiment, the highest dose was dubbed the "100%" dose, the middle dose the "10%" dose, and the lowest dose as the "control" dose.

The second independent variable was the type of organism whose disinfection was being evaluated. Organism type varied between four possible values: none (as a negative control), *Bacillus cereus* spores, *Escherichia coli* "B" strain, and MS2 male-specific (F+) coliphage. The initial dose of these organisms, or the "disinfection challenge" posed by these organisms, was high enough to enable a potential 4 $\log_{10}$ (99.99%) reduction to be measured by our assay techniques. Full details of the reason for the choice of these organisms are given elsewhere in "Materials and Methods".

The third independent variable was the type of test water; two types of test waters were used in these experiments. The first was a sterile, buffered, heavy-metal-demand-free (HMDF, or de-ionised) test water of pH roughly 7.5, and the second was identical to the first save for the inclusion of natural organic matter (NOM) as discussed elsewhere in "Experimental Approach" section. Fuller characterisation of the lake water was performed; details are given elsewhere in "Materials and Methods".

Dependent Variables: Sampling and Assays.

There were two overall dependent variables measured during the course of this experimentation. The first was the measurable concentration of culturable microbes, taken at various time points over the course of each disinfection trial. The time points used were 0, 20, 60, 180, and 360 minutes (0, 0.33, 1, 3, and 6 hours), and the concentration of culturable microbes at each time point was quantified as "colony-forming units" (CFUs) per unit volume or "plaque-forming units" (PFUs) per unit volume for bacteria and viruses respectively.

The number of CFUs and PFUs per unit volume was quantified using standard microbiological assays as described elsewhere in "Experimental Approach". The second dependent variable was the measurable concentration of disinfectant, taken initially (at time t = 0 minutes) and at the conclusion of the disinfection trial (at time t = 360 minutes). The measurable concentration of the disinfectant was quantified via inductively-coupled plasma mass spectrometry (ICP-MS); full details of the quantification of culturable microbes over time, as well as the quantification of the initial and final amounts of available disinfectant are given elsewhere in "Materials and Methods".

Evaluation of Disinfection Efficacy: The World Health Organisation Performance Targets

For the purpose of this experimentation, an adequate performance of disinfection was considered to meet or exceed the performance targets set by the World Health Organisation in its document "Evaluating Household Water Treatment Options: Health-Based Targets and Microbiological Performance", published in 2011. For gram-negative enteric bacteria, modelled here by *Escherichia coli* "B" strain, as well as for protozoan parasites and other more resistant drinking-water pathogenic organisms, modelled here by *Bacillus cereus* spores, the recommendation is that household water treatment systems (HWTS) must meet at least a 99% (2 $\log_{10}$) reduction in

culturable organisms as a "protective" level of performance, if additional human health data from epidemiological studies provides supporting field evidence that such treatment systems are effective in context to protect against waterborne disease. For enteric viruses, modelled here by MS2 coliphage, the "protective" performance target is at least a 99.9% (3 $\log_{10}$) reduction in culturable organisms.

IX. Materials and Methods

Preparation

Prior to experimentation, selective assay agar (Awesome Agar) plates were prepared. Per 500 millilitres of agar medium, 20 grams of Difco Trypticase Soy Agar, 15 milligrams of Neutral Red, and 5 grams of lactose were added. The mixture was autoclaved for 20 minutes, vented, mixed well and used to pour agar plates (with approximately 15 millilitres of agar used per plate). Plates were dried at 45 degrees centigrade for 24-36 hours and placed in refrigerated storage prior to use.

All batch disinfection experiments were conducted in sterile 50 mL polypropylene (PP) tubes. Storage of test waters, storage of sample tubes, and further sample dilutions were all performed in tubes of lime glass. Test waters were prepared by aseptically adding 10 grams of HEPES buffer per 1 litre of deionised water and mixing thoroughly. For water intended to contain dissolved organic matter, an additional 10 millilitres of stock Catfish Lake water containing 10 mg of additional dehydrated NOM was added per litre. The solution was subsequently autoclaved for 20 minutes, allowed to cool to room temperature, and stored at room temperature overnight under conditions of darkness. "Balls!" and "ass!" were periodically exclaimed as laboratory workers came into contact with heated surfaces. In fact, they were pretty much exclaimed at every step of these experiments.

Immediately prior to experimentation, the stock colloidal solutions of CuO and ZnO were agitated until visual homogeneity was achieved; they were further sonicated at 90% of maximum intensity using a Lab-Line Sonicator, Model 9100 with Tip Model Number MT1-1613 (Melrose Park, Illinois) for five minutes prior to use. The concentration of each nanoparticle stock solution was calibrated to be 2 grams per litre. This was to ensure that an addition of a relatively small amount of stock nanoparticle suspension was sufficient to adequately dose a 50 millilitre reactor vessel volume.

Test waters were divided into two sets prior to experimentation, of which one was intended to be organism-positive and one was intended to remain organism-free. Five millilitres of *B. cereus* spore stock containing a total of $5*10^7$ spores were added per 500 millilitres of organism-positive test water, along with 1.5 millilitres of log-phase, washed, and re-suspended *E. coli* culture (an amount experimentally determined to contain $5*10^7$ CFUs) prepared following standard methods (see Armstrong, 2011; Komandur, 2011; Malone, 2012).

The phage suspension added was obtained as frozen stock with approximate infectivity titer of $1*10^{12}$ PFU per millilitre, which was then diluted ten-thousand-fold in DPBS, and then passed through serial 2 and 0.08 micrometre pore size "Whatman Nucleopore Track-Etch" polycarbonate membrane filters (approximately 2 cm in diameter) that had been pre-washed with 0.1% Tween-80 solution and then pre-rinsed with heavy-metal-demand-free (de-ionised) waters. A 0.5 millilitre volume of this diluted MS2 filtrate was added to1 litre of test water. This step was performed in order to eliminate aggregates of virus particles from the test water. Approximate titre targets for all microorganisms were $5*10^7$ culturable microorganisms per litre, and were selected to be well within the detection limits ($5*10^8$ culturable microorganisms at the high end and $5*10^6$ culturable microorganisms at the low end) of this experimentation.

Test waters were dispensed amongst reactor vessels as shown below in Table 1, tested to ensure appropriate pH, and initially sampled for 0-minute data. They were subsequently dosed with metal additives according to their respective assigned concentrations.

Organism-Positive Test Waters	Organism Negative Test Waters
Vessel 1 (0.2 mg/L metals concentrations) 5 µL of CuO Nanoparticle Stock Suspension 5 µL of ZnO Nanoparticle Stock Suspension With Organisms	Vessel 4 (0.2 mg/L metals concentrations) 5 µL of CuO Nanoparticle Stock Suspension 5 µL of ZnO Nanoparticle Stock Suspension No organisms
Vessel 2 (2 mg/L metals concentrations) 50 µL of CuO Nanoparticle Stock Suspension 50 µL of ZnO Nanoparticle Stock Suspension With Organisms	Vessel 5 (2 mg/L metals concentrations) 50 µL of CuO Nanoparticle Stock Suspension 50 µL of ZnO Nanoparticle Stock Suspension No organisms
Vessel 3 (metals free-control) No metals With Organisms	[redacted]

Table 1: Reactor Vessels and Metal Additive/Microorganism Dosing

The time was recorded upon the dosing, and used as a reference to calculate the following sampling times. At each sampling time, including the initial 0-minute sampling, a 500 microlitre sample was taken from reactor vessels 1-3 and added to the corresponding dilution tube with 4.5 millilitres of diluent (DPBS) to make an initial ten-fold dilution. Serial 10-fold dilutions (also in DPBS) were performed in between sampling times, and were performed until a final dilution of 10^{-4} was achieved.

At 0 and at 360 minutes, samples were taken for ICP-MS analysis. Separate sterile 5 millilitre pipettes were used to take 3 millilitre samples from vessels 1-6 (with 6 being the control vessel, denoted by the blacked-out box above). These samples were dispensed into sterile 15 millilitre PP centrifuge tubes. Microorganism-positive samples were centrifuged at 3000 rpm for 15 minutes and decanted, in order to avoid analytical errors associated with aggregated particles, such as larger microorganisms, being present in the sample. It is possible that such centrifugation removed metal nanoparticles bound with removed particles; however, the unbound metal nanoparticles used in experimentation here were sized such that removal by centrifugation under these

conditions would be highly unlikely (Bodnar, 2011). Centrifuge tubes were subsequently labelled, and analysed.

Sample dilutions prepared during the course of the experiment were subsequently assayed for bacterial colony-forming units (CFUs) and viral plaque-forming units (PFUs). From each sample dilution, at each time point, two separate samples of 100 microlitres were pipetted onto two separate Awesome Agar plates. The samples were spread using sterile plastic "hockey sticks" and allowed to dry. After drying, the plates were covered, turned upside down, and allowed to incubate at 37 degrees Centigrade for 24 hours prior to counting colonies. Infectivity assay for MS2 PFU counts were performed by the single agar layer plaque assay following the U.S. EPA Standard Method 1602.

Methods of Statistical Analysis

Confidence Intervals and Limits of Significance

With no exceptions, throughout the course of all statistical analysis and modelling performed as part of this research, standard values for confidence intervals and limits of significance were used. All intervals correspond to a 95% level of confidence, indicating that the approximation of a repeated sampling distribution to the normal distribution curve (the "bell curve") is valid and that upon such repetitive sampling, we can be confident that 95% of values returned predicated upon our experiment data would fall within the indicated range. All limits of significance correspond to an alpha-level of 0.05; this alpha value indicates that the probability that our experimental data were yielded in a normative scheme, the assumptions of which corresponded with null hypothesis in question, was less than 5%.

Standardisation of Dilution and Experimental Duplicate

Different CFU/PFU counts were reported per dilution. One number, representing total number of CFUs per unit volume per time point per vessel, was used for further analysis. Counts were therefore summed across all dilutions, and divided by the total units of volume assayed. Units of volume could be arbitrarily assigned, if conventions were kept consistent throughout the analysis. For example, counts summed across dilutions of 10^{-2}, 10^{-3}, and 10^{-4} would be divided by a sum of the units of volume from which those counts were derived. If a 10^{-2} dilution were considered 1 unit of volume, subsequent dilutions would be 0.1, and 0.01 units respectively. Counts summed across dilutions of 10^{-2}, 10^{-3}, and 10^{-4} would therefore be divided by a factor of 1.11 prior to further analysis.

At each time point, two samples were taken. Upon dilution standardisation, these counts were averaged to yield one single count per vessel per time point. These single counts were used for further analysis.

Steady-State Control Count Verification, Standardisation in Aberrant Cases, and Final Calculation of Log (N_T/N_0)

Once counts had been standardised for dilution and sample assay duplication, the survival ratio N_T/N_0 was calculated. Counts for 0 minutes, taken prior to addition of any disinfectant, were considered "N_0", or the initial microorganism population for that particular vessel. Subsequent counts across time points ("N_T") were therefore divided by the initial count corresponding to their vessel to calculate the survival ratio. These N_T/N_0 values were the original inputs for non-first-order kinetic analysis, using the One-Hit Two-Population (OHTP) model and further analysis was performed to obtain the inputs for Chick-Watson and Hom analysis, as described below.

Prior to such statistical and/or kinetic analysis, the assumption of a steady-state control vessel for microbe concentration required verification. Without a steady-state microbe concentration in the control vessel, survival ratios would have to be standardised against any systematic increase/decrease observed in the microbe concentration in the control, to account for effects induced by statistically significant microbial population fluctuation. In order to verify a steady-state control vessel microbial concentration, the N_T/N_0 values for that control were statistically compared to an "ideal" control of no variation across time. The null hypothesis of no variation in this control sample was tested against the alternative hypothesis of variation holistically, across all time points. Statistically insignificant test results were taken to indicate a steady-state control vessel; statistically significant test results were taken to indicate that further standardisation was needed in order to eliminate the confounding effects of microbial population change over time.

For those cases in which verification of a steady-state control vessel failed, further standardisation was performed against the control counts. The N_T/N_0 (survival) ratios calculated for each vessel were divided by the N_T/N_0 of the control vessel at each particular time point. This resulted in a standardised data set in which initial population counts for each vessel had a survival ratio of 1, and the N_T/N_0 for the control counts did not deviate from their initial survival ratio throughout the course of the experiment.

At this point, the base-10 logarithms of the N_T/N_0 values were taken, in order to facilitate log-linear analysis. The $\log_{10}(N_T/N_0)$ values were used for first-order kinetic analysis (the Chick-Watson model) in addition to a commonly applied non-first-order deviation from Chick-Watson kinetics, the Hom model.

Methods of Kinetics Modelling and Mixed-Model Analysis

The Chick-Watson Model

The Chick-Watson model is a standard model of exponential decay, in which the population at any point in time varies in proportion with time and a rate constant as shown below:

$$N_T = N_0 * e^{-kt}$$

In the equation above, N_T is the population at any point in time, N_0 is the initial population, e is Euler's constant, k is a rate constant that reflects the concentration of the disinfectant and the rate of exponential decay, and t is time.

In order to fit experimental data to the Chick-Watson model, it was algebraically modified to yield a first-order equation, shown below:

$$Log\left(\frac{N_T}{N_0}\right) = -k * t$$

This model was applied to all the data using simple least-squares regression. For all sets of data that yielded non-zero disinfection, two further models were applied, that did not assume first-order decay kinetics.

The Hom Model

The Hom model is a standard variation of the Chick-Watson model that attempts to take into account random variation in culturable microorganisms over time by assigning an additional parameter, m, to the time factor as shown below. Values of m that are greater than one correspond to "shouldering" kinetics, i.e. kinetics that display an initial lag time prior to ideal Chick-Watson decay. Values of m that are less than one correspond to "tailing" kinetics, i.e. kinetics that display

initial Chick-Watson decay but taper off after a certain amount of time has passed. Note that the Hom model, by definition, will always provide a fit at least as suitable, if not more, than the standard Chick-Watson model, since a value of *m* equal to 1 simplifies the model to the original Chick-Watson equation. To wit:

$$N_T = N_0 * e^{-k*t^m}$$

In order to fit experimental data to the Hom model, it was algebraically modified to a log-linear expression as shown below:

$$Log\left(\frac{N_T}{N_0}\right) = -kt^m$$

This model was applied to the data using JMP™10 Pro, a statistical software suite published by the SAS company. The two parameters in the model, *k* and *m*, were initially calculated and used to build a predicted data set. Mean Squared Error and Root Mean Squared Error associated with the model were also reported. To verify the model, and in order to arrive at a correlation coefficient, the predicted data set was subsequently compared with the actual data set via a matched-pairs t-test.

The One-Hit Two-Population Model

The One-Hit Two-Population model (OHTP) is a standard non-first-order kinetics model used when analysing disinfection data. It fits data according to the assumption that the disinfection response of the target population of microorganisms is comprised of two sub-populations, that respond to the disinfectant differently. Each sub-population is treated differently in the kinetics analysis, which follows the formula below:

$$\frac{N_T}{N_0} = fe^{-k_1*t} + (1-f)e^{-k_2*t}$$

In the above equation, f is considered the fraction of the original population that is more susceptible to the disinfectant; the quantity (1-f) corresponds to the fraction of the original population that is less susceptible. The rate constants k_1 and k_2 are analogous to the rate constants used in the Chick-Watson and Hom models.

This model was also applied to the data using JMP™9 Pro. The three parameters in the model, k_1, k_2, and f were initially calculated and used to build a predicted data set in much the same fashion as in the Hom model. However, limits were placed on these parameters. The fraction f was not allowed to decrease below 0, or increase above 1, and the rates of exponential decay k_1 and k_2 were not allowed to be greater than 0, as that would indicate a situation where one population was being actively disinfected and another was actually increasing in number throughout the course of the experiment. Under these experimental conditions and given that results for samples with nanoparticles were normalized against nanoparticle-free control samples that were corrected for microbial increases over time, such results would be unlikely.

As a non-first-order model, a lack of mathematical convergence was a potential outcome for the One-Hit Two-Population model when applied to any real-world data. This means that, within the parameter limits placed on the model (as described above), the algorithm used by the modelling software may not arrive at a "solution" (taken to mean a real-world non-imaginary numerical set of projected data within the range of numbers found during experimentation) within a certain number of attempts. The number of attempts is chosen by the researcher, and is set at a value that indicates to a reasonable degree of certainty that the model is "stuck" and unable to proceed to a solution. In all cases, the limit on the number of iterations for model convergence was set at 10,000 attempts.

When these conditions were placed, in all cases k_2 was found to be zero. In other words, the model considered one sub-population entirely unaffected by the disinfectant, with all observed disinfection occurring in the other sub-population. In this case, the fraction *f* corresponds to that fraction of the initial population against which the disinfectant proved efficacious. In all cases, the rate constant k_1 was associated with the susceptible population. For this reason, the One-Hit Two-Population model was not used for further extrapolation. To verify the model, and in order to arrive at a correlation coefficient, a predicted data set was subsequently compared with the actual data set via a matched-pairs t-test.

Extrapolation from Obtained Data

The models calculated for each data set were used for extrapolation, in order to determine the length of time required, under any particular experimental combination, to reach the W.H.O. performance target values of 2 $\log_{10}$ (99%) reduction of bacteria and protozoan parasites and 3 $\log_{10}$ (99.9%) reduction of viruses for drinking-water treatment technologies intended for POUH applications (W.H.O., 2011)

By definition, the Hom model provides a curve whose fit is at least as good as, if not superior to, that of the Chick-Watson model. Also by definition, the One-Hit Two-Population model cannot be used for extrapolation to reach performance targets, owing to a residual fraction left unaffected by the disinfectant (i.e. one that had a rate constant, k_2, of 0). All extrapolation was therefore performed using the Hom model. The following equations to calculate the time required to reach 2 $\log_{10}$ (99%) and 3 $\log_{10}$ (99.9%) reductions were $t = \left(\frac{2}{k}\right)^{\frac{1}{m}}$ and $t = \left(\frac{3}{k}\right)^{\frac{1}{m}}$. The first was applied to *B. cereus* spores and *E. coli* and the second for MS2 bacteriophage.

Mixed-Model Analysis

Motivation

For the purpose of analysing the effect of metal nanoparticle dose difference, or the presence of natural organic matter (NOM) in buffered test water, cross-model analysis was pursued in addition to the standard matched-pairs t-test employed previously. Though valid, the matched-pairs t test suffers from overbroad margins of error in the case of data sets with low sample size. All data sets analysed in this research consisted of five data points, leading to large margins of error expressed and low generalizability when matched-pairs t-tests were used to analyse the significance of the low versus the high metal additive dose, or the significance of synthetic versus NOM-amended waters. Accordingly, cross-model parametric analysis was performed between data sets differing by only a single variable (e.g., metal dose, or presence of NOM in water). Two different parametric techniques were employed, for linear and non-linear cases.

Linear Cases

Whenever possible, baseline parametric analysis is the preferred method for cross-model comparisons. Associated error with baseline parametric analysis becomes large as the number of variables analysed increase; for this reason, its use is typically constricted to the case of univariate analysis. In the case of this research, this analysis consisted of arbitrarily assigning one data set to the role of "baseline", and the data set with which it must be compared, the role of "test". In the case of the testing of dose significance, the 0.2 milligram per litre dose was assigned to be the "baseline" data set, and the 2 milligram per litre dose was assigned to be the "test" set. In the case of the testing of NOM significance, the waters without NOM were assigned to be the "baseline" data sets, and the waters with NOM were assigned to be the "test" set. Assignments were made in order to

frame the significant effects (if any) of dose and NOM in terms of their magnitudes of difference from results for baseline conditions. .

The "baseline" data set was fit with the classic log-linear Chick-Watson model and designated as a function of t, as shown below:

$$f_{baseline}(t) = -k * t$$

and the rate constant k noted. Subsequently, the "test" data set was fit with the baseline parameters, and an additional parameter β, the target of significance testing, as shown below:

$$f_{test}(t) = f_{baseline}(t) + \beta * t$$

When fit, the test function yielded a 95% confidence interval for the estimate of β. A statistically insignificant result was yielded if the 95% confidence interval included 0, and vice versa. If the value for β is negative, that indicates that (with respect to the y-axis) the "test" result exhibits a significant decrease from the "baseline". For the purpose of this research, this implies an increase in disinfection efficacy. If the value for β is positive, that indicates a significant increase from the "baseline" with respect to the y-axis. In this research, this implies a decrease in disinfection efficacy. In this research, the linear case applied to all situations involving *B. cereus* and *E. coli*.

Non-Linear Cases

When baseline parametric analysis cannot be accurately performed, as is often the case in models resulting from data sets with low n, multiple parameters of fit, or (in this case) both, analysis of parametric joint confidence spread can be used. This well-established technique is discussed in detail elsewhere (e.g. Box et al, 1978); essentially, the spread of the 95% confidence intervals of model parameters are compared between models and their regions of intersection (if any)

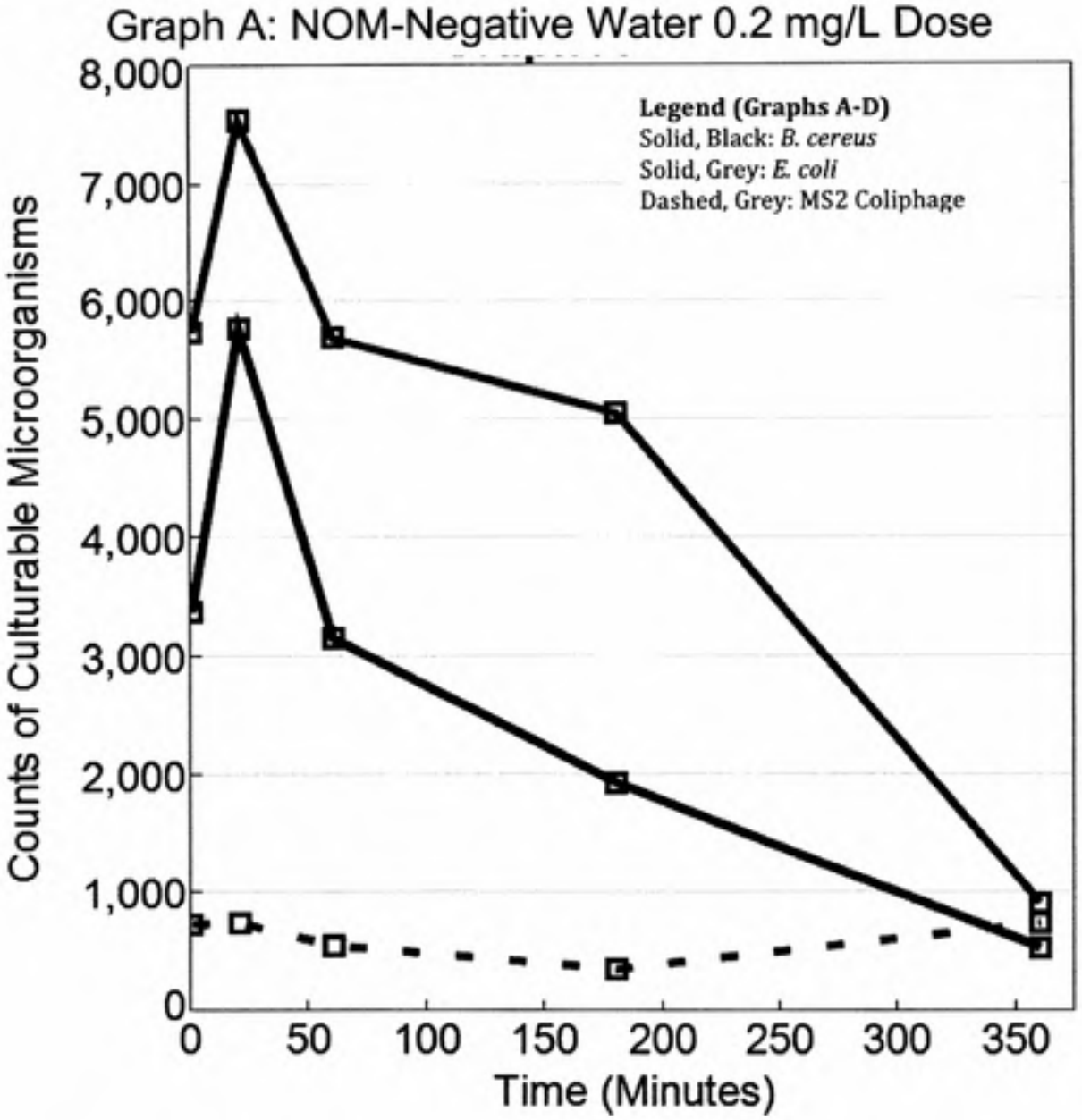
identified. Intersection of the confidence intervals of every single parameter is required for two models to have a statistically insignificant difference; therefore, following the principle of the "one-check fail test", even a single deviant parameter implies a statistically significant difference exists between the models.

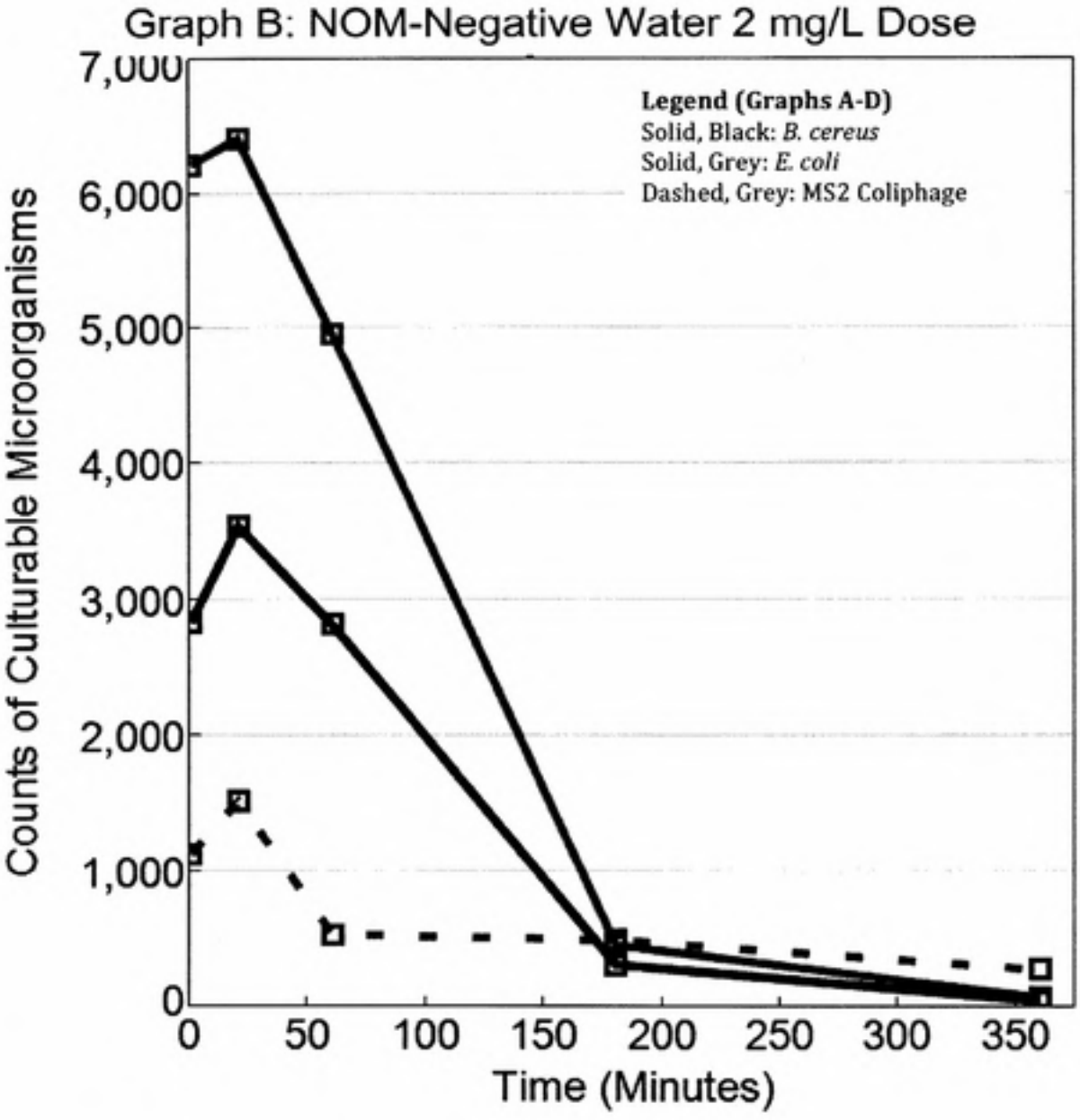
In this research, such analysis was performed only in the case of MS2 coliphage; for those purposes the Hom model was used. Accordingly, two parameters were compared, k, and m. If the 95% confidence intervals yielded no degree of overlap between the k of one data set and the k of another, or between the m's of the two data sets, the variable tested was considered statistically significant under the given experimental conditions.

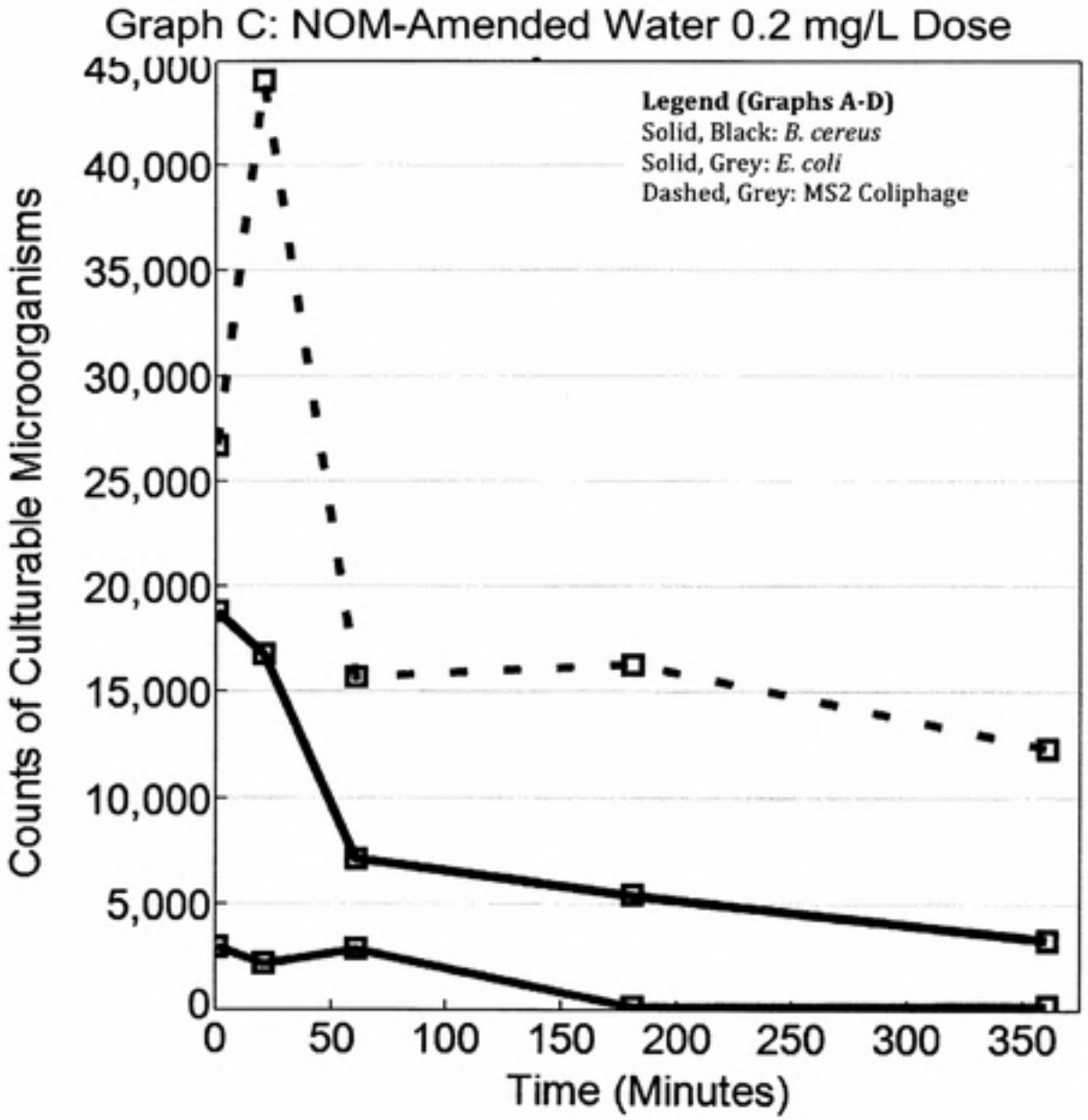
X. Results

Key Results

The graphs below represent the overall changes in raw, unanalysed counts of culturable microbes over time in the different test waters with different metal oxide nanoparticle doses.




Graph B: NOM-Negative Water 2 mg/L Dose
Legend (Graphs A-D)
Solid, Black: B. cereus
Solid, Grey: E. coli
Dashed, Grey: MS2 Coliphage
Counts of Culturable Microorganisms
7,000
6,000
5,000
4,000
3,000
2,000
1,000
0
0
50
100
150
200
250
300
350
Time (Minutes)


Graph C: NOM-Amended Water 0.2 mg/L Dose
Legend (Graphs A-D)
Solid, Black: B. cereus
Solid, Grey: E. coli
Dashed, Grey: MS2 Coliphage
Counts of Culturable Microorganisms
45,000
40,000
35,000
30,000
25,000
20,000
15,000
10,000
5,000
0
0
50
100
150
200
250
300
350
Time (Minutes)

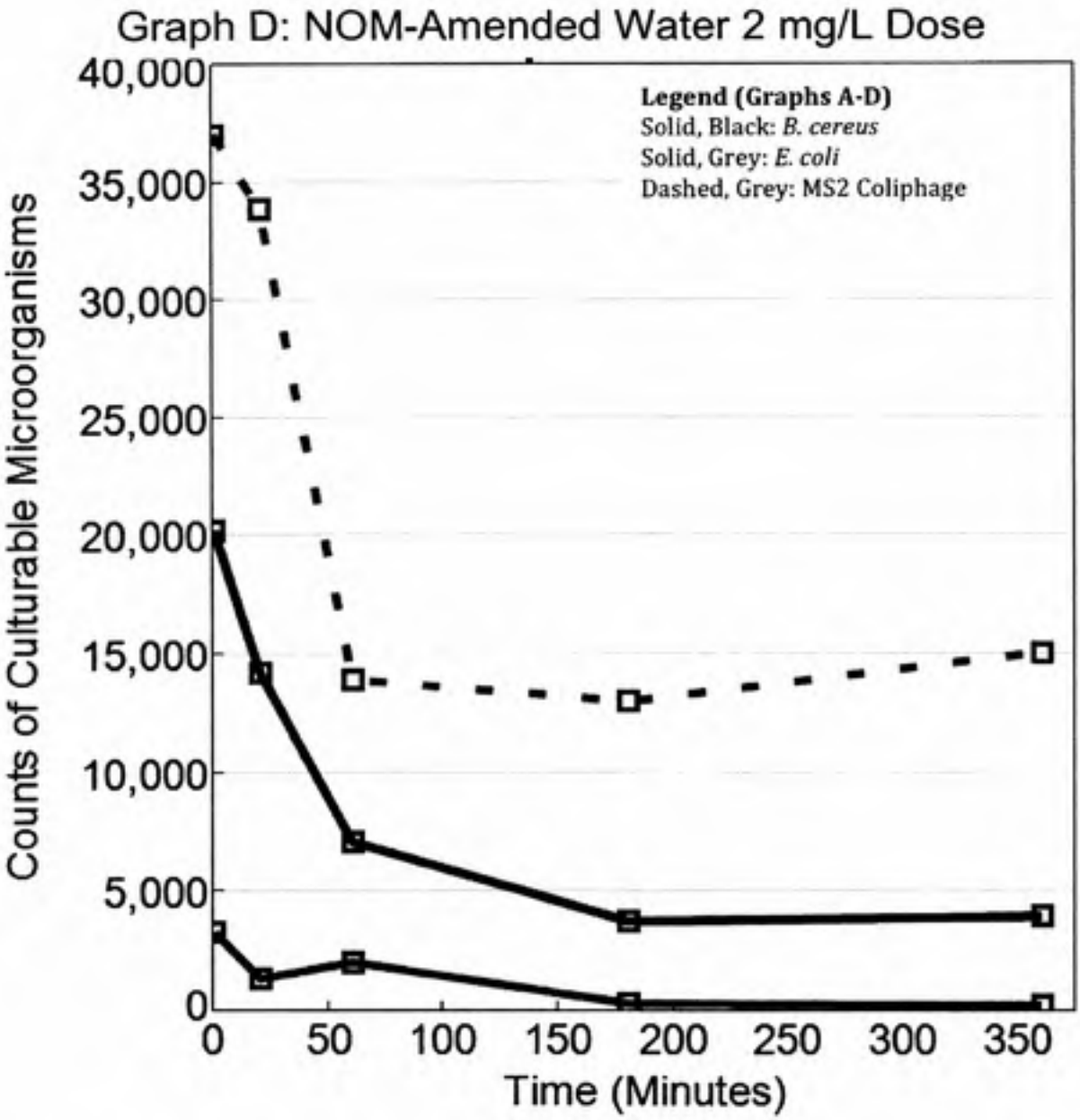


Figure 1: Overall reductions in culturable microbes by exposure to copper and zinc oxide nanoparticles over the course of 360 minutes. Graph A—0.2 mg/L metals doses in buffered water. Graph B—2 mg/L metals doses in buffered water. Graph C—0.2 mg/L metals doses in NOM-amended buffered water. Graph D—2 mg/L metals doses in NOM-amended buffered water.

An overall decrease in numbers of culturable microbes over time can be observed under all conditions tested (Figure 1). However, certain time-point-by-time-point aberrances were noted between measurements taken at 0 minutes and 20 minutes. In particular, certain data sets

displayed a moderate increase in culturable organisms in the first 20 minutes of the experiment, an observation that confounds one of the central assumptions of disinfection kinetic analysis; that overall numbers of organisms, if they are not inactivated by the disinfectant, will remain constant. In order to assess the potential significance of these findings, a two-sample t-test was performed between data from 0 minutes and data from 20 minutes for those data sets which exhibited such an increase. Those data sets, and the significance of the findings, are presented below.

Data Set	Two-Sample T Test p Value	Statistically Significant at α=0.05?
Synthetic *Bacillus* 0.2 mg/L	**0.0314**	**Yes**
Synthetic *Bacillus* 2 mg/L	0.4837	No
Synthetic *E. coli* 0.2 mg/L	**0.0044**	**Yes**
Synthetic *E. coli* 2 mg/L	0.7945	No
Organic *Bacillus* 0.2 mg/L	0.6949	No

Table 2: Statistical Analysis and Significance of Test Conditions that Exhibited Increased Culturable Organisms by 20 Minutes Compared to Time 0

Given the lack of significant difference between the pool of 20-minute measurements and the pool of 0-minute measurements for three of the above data sets (Synthetic *Bacillus* 2 mg/L, Synthetic *E. coli* 2 mg/L, and Synthetic MS2 0.2 mg/L), t = 20 minute $\log_{10}$ reduction values were capped at the t=0 value in all further data analysis, for the purpose of reducing error in extrapolation at later time points. For those two data sets (Synthetic *Bacillus* and *E. coli* 0.2 mg/L) that exhibited statistically significant increases between the two time points, $\log_{10}$ reduction values were left as they were for all time points.

In order to standardise the data and enable cross-experimental comparisons, the data analysis strategy described above was applied to the experimental results. The final results, the $\log_{10}$ reductions observed over the course of 360 minutes, for all experiments are summarised below in Figures 2-5

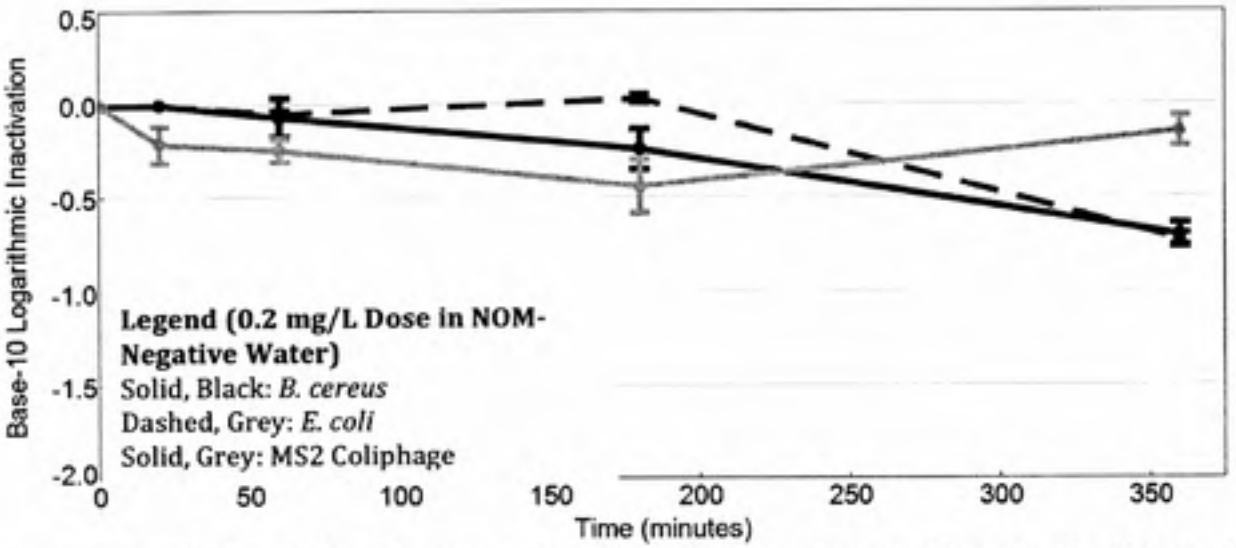


Figure 2: Base-10 Logarithmic Inactivation Curves of *B. cereus* Spores, *E. coli*, and MS2 Coliphage by the 0.2 mg/L Metal Additive Dose in NOM-Negative Water (Error Bars +/- 1 S.E.)

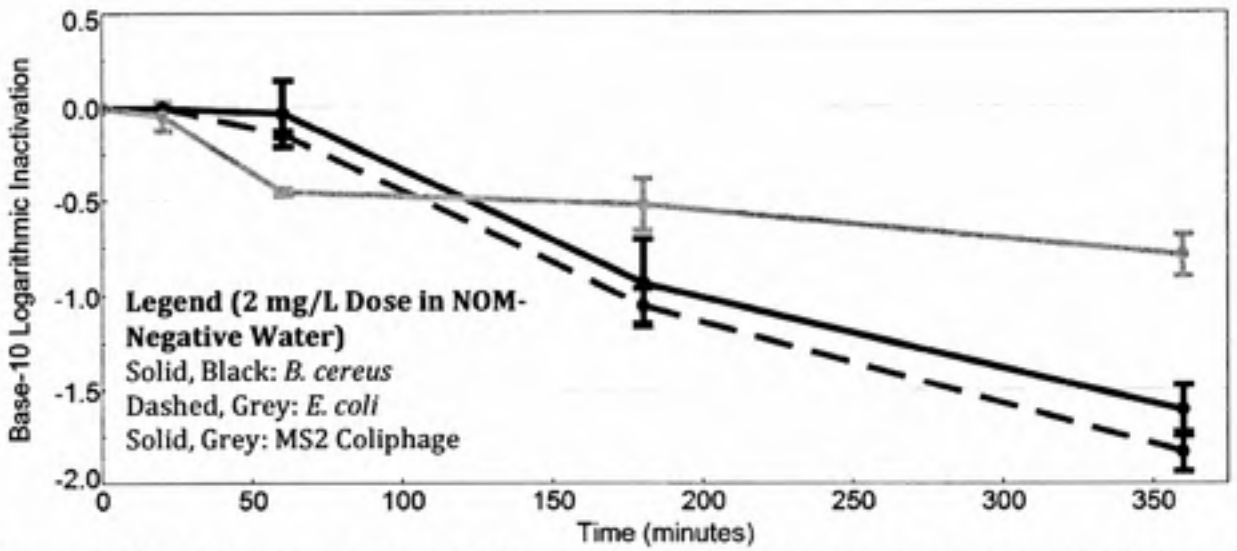


Figure 3: Base-10 Logarithmic Inactivation Curves of *B. cereus* Spores, *E. coli*, and MS2 Coliphage by the 2 mg/L Metal Additive Dose in NOM-Negative Water (Error Bars +/- 1 S.E.)

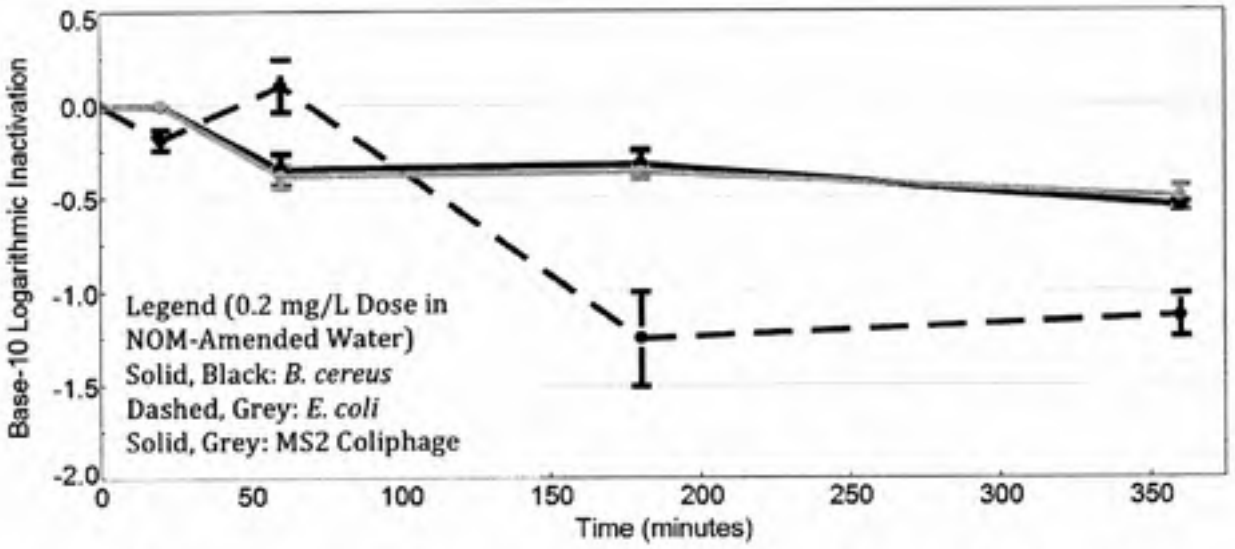


Figure 4: Base-10 Logarithmic Inactivation Curves of *B. cereus* Spores, *E. coli*, and MS2 Coliphage by the 0.2 mg/L Metal Additive Dose in NOM-Amended Water (Error Bars +/- 1 S.E.)

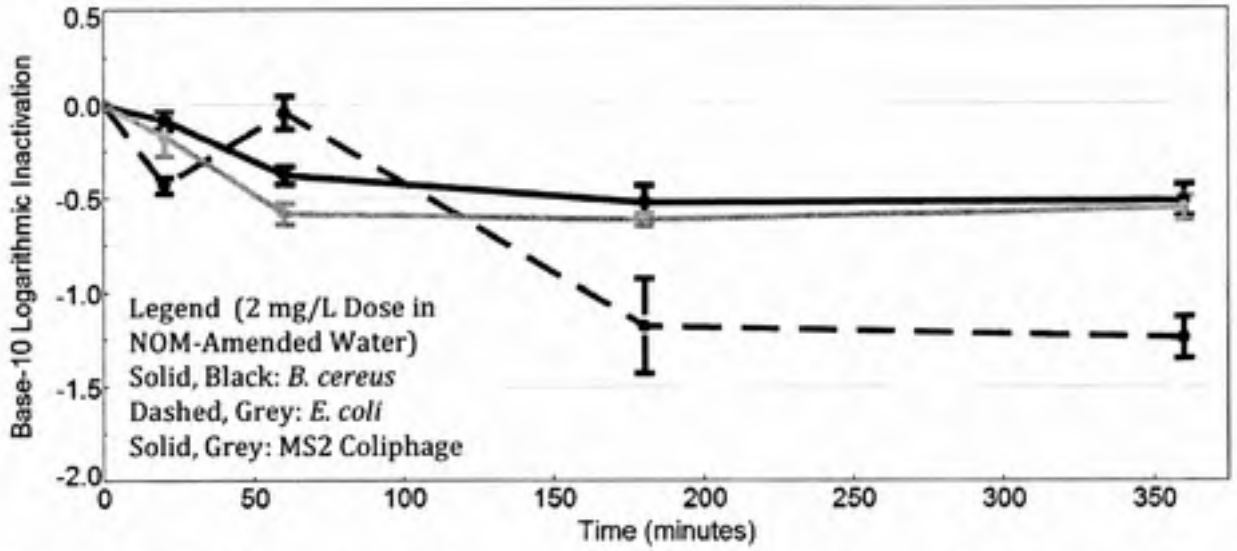


Figure 5: Base-10 Logarithmic Inactivation Curves of *B. cereus* Spores, *E. coli*, and MS2 Coliphage by the 2 mg/L Metal Additive Dose in NOM-Amended Water (Error Bars +/- 1 S.E.)

As is shown above in Figures 2-5, the vast majority of experimental conditions yielded $\log_{10}$ reductions after 360 minutes of contact time in the range of 0.5 to 0.75, corresponding to an approximate 33% to 85% reduction in absolute numbers. Only two experimental conditions (both

doses against *E. coli* in NOM-amended waters (Figures 4 and 5) yielded log_{10} reductions in the range of 1 (90%), and only two (the 100% dose against *Bacillus* and *E. coli* in synthetic waters, Figure 3) yielded log reductions between 1.5 and 2 (99%). Two experimental conditions, specifically those for MS2 coliphage at the 0.2 mg/L dose of metal nanoparticles in both NOM-negative and NOM-amended water (Figures 2 and 4) gave no significant log_{10} reductions. The array of experimental combinations and their final log reductions are presented below (full statistical output may be found in the appendix).

Experimental Conditions	0.2 mg/L Dose	2 mg/L Dose
Synthetic Water // *B. cereus*	0.79	1.69
Synthetic Water // *E. coli*	0.80	1.87
Synthetic Water // MS2	Not significantly different from 0	0.78
Organic Water // *B. cereus*	0.54	0.49
Organic Water // *E. coli*	0.98	1.05
Organic Water // MS2	Not significantly different from 0	0.53

Table 3: Experimental Conditions of Test Microbe, Test Water and Metal Dose and Resultant Log_{10} Reductions of Culturable Microbes after 360 Minutes Contact Time

Further data analysis was not performed on those combinations yielding no significant disinfection (i.e., a 0.2 mg/L dose used against MS2 bacteriophage, in water both with and without dissolved organic matter).

Statistical Analysis

Before performing kinetics modelling on the data, certain statistical questions required resolution. Normally, over time, microbe counts obtained from sampling a control (metal-free) vessel fluctuates about the norm. Occasionally, for reasons that are not clear, microbe control counts trend over time towards either a net increase or a net decrease. As explained above, in order to account for such systematic variation of the metals-free control counts, the microbe counts of the metals-treated samples were standardised to the control count for their respective time points prior to performing logarithmic analysis expressing the metal-treated sample (N_t) divided by the metal-free

control sample count (N_0) as the expression for the survival ratio (and thereby magnitude of disinfection).

For these data, the reliability of the assumption of a control vessel having a steady-state microbe count required evaluation prior to microbial reduction kinetics modelling. The validity of this assumption was tested with a matched-pairs t-test of the data for the microbial control counts, performed on the array of N_{CT}/N_0 ratios, where N_{CT} is the concentration in the metal-free control sample at any time, T. N_{CT}/N_0 values for the control vessel were treated as one set of samples, and were compared to a set of control sample of a perfect series of N_{CT}/N_0 values, i.e. one where the value of N_{CT}/No remained at "1" over time. Those data sets that resulted in a significant difference from the ideal were subjected to an additional control standardisation prior to logarithmic analysis. These results are presented below (full statistical output may be found in the appendix).

Experimental Conditions	**Matched-Pairs T-Test p Value**	**Statistically Significant/ Additional Standardisation?**
Synthetic Water // *B. cereus*	0.7235	No
Synthetic Water // *E. coli*	0.4023	No
Synthetic Water // MS2	**0.0209**	**Yes**
Organic Water // *B. cereus*	**0.0459**	**Yes**
Organic Water // *E. coli*	0.6384	No
Organic Water // MS2	**0.0177**	**Yes**

Table 4: Matched-Pairs T-Test Results for Control Vessel N_T/N_0 under Various Experimental Conditions

Kinetics Modelling of Disinfection

The data for microbial reductions over time were subjected to kinetics modelling. All 12 data sets were subjected to curve-fitting following the Chick-Watson model, and all 10 data sets that yielded non-zero disinfection results were subjected to curve-fitting following the Hom and One-Hit Two-Population models. The overall fit of the Chick-Watson model was evaluated using a combination of the R^2 value obtained from the linear fit of the Log (N_T/N_0) microbe reduction values over time (in

minutes), and an examination of the data to identify apparent non-linear trends. The fit of the Hom and One-Hit Two-Population Models were obtained from a non-linear least-squares regression R^2. Those instances where the One-Hit Two Population model failed to converge (as described in the "Materials and Methods" section, subsection "Methods of Kinetics Modelling and Mixed-Model Analysis") are denoted with a dash. The results are presented below.

Experimental Conditions	Chick-Watson Fit	Hom Fit	OHTP Fit	Model Used
Synthetic Water // *B. cereus* // 0.2 mg per L Dose	96%	99.99 %	-	Hom
Synthetic Water // *B. cereus* // 2 mg per L Dose	97%	98%	-	Hom
Synthetic Water // *E. coli* // 0.2 mg per L Dose	83%	100%	-	Hom
Synthetic Water // *E. coli* // 2 mg per L Dose	97%	99%	97%	Hom
Synthetic Water // MS2// 0.2 mg per L Dose	5%	N/A	N/A	N/A
Synthetic Water // MS2 // 2 mg per L Dose	83%	96%	97%	Hom
Organic Water // *B. cereus* // 0.2 mg per L Dose	77%	92%	93%	Hom
Organic Water // *B. cereus* // 2 mg per L Dose	**65%**	**94%**	**99%**	**OHTP**
Organic Water // *E. coli* // 0.2 mg per L Dose	73%	88%	91%	Hom
Organic Water // *E. coli* // 2 mg per L Dose	81%	94%	88%	Hom
Organic Water // MS2// 0.2 mg per L Dose	66%	N/A	N/A	N/A
Organic Water // MS2 // 2 mg per L Dose	**44%**	**89%**	**99%**	**OHTP**

Table 5: Curve-Fitting Validity for Models Applied to Various Experimental Conditions of Disinfection

By definition, the Hom model's fit will be at least equal to, if not greater than, the Chick-Watson fit. The Hom model usually yielded the best curve-fitting results, providing results markedly inferior to that of the One-Hit Two-Population model in only two of the twelve data sets, namely that of *B. cereus* and MS2 coliphage treated with a 2 milligram per litre dose of the metal additive in waters

with NOM (bolded). Graphical representation of the Hom model fits for all the data save for the two cases highlighted above are presented below.

For the two cases bolded above, the One-Hit Two-Population model is shown. The x-axis for all models shown below denotes time in minutes. Relevant model parameters for the Hom model in eight of the ten analysed data sets, and for the One-Hit Two-Population Model in the remaining two data sets, are also presented below. All parameters are limited to three significant figures.

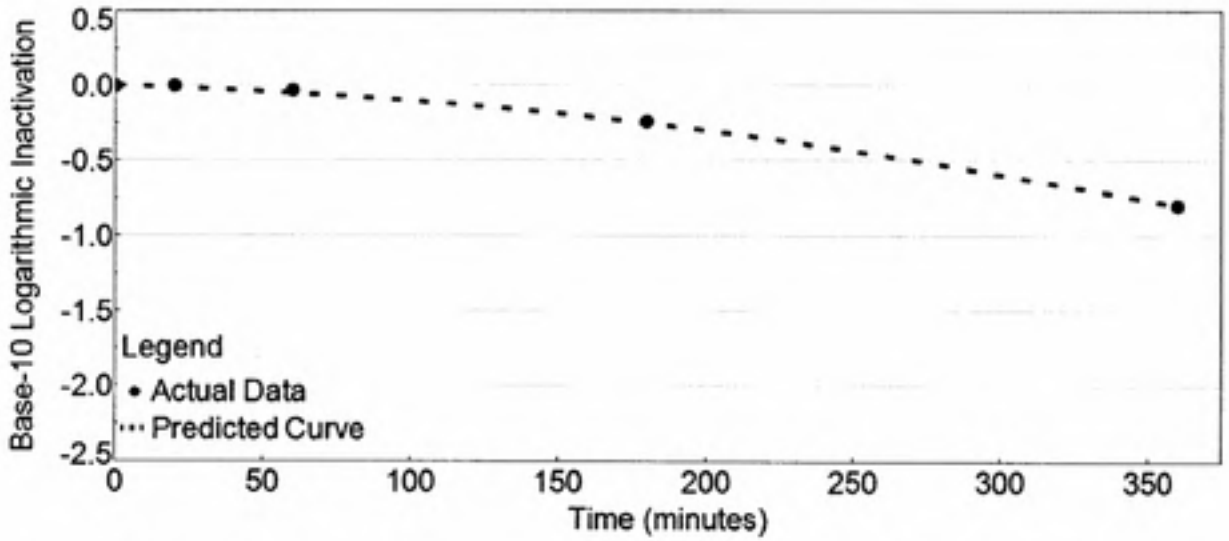


Figure 6: Shouldering Curve Fit by the Hom Model of the Disinfection of *Bacillus cereus* Spores in NOM-Negative Waters by 0.2 mg/L Metal Additive Dose

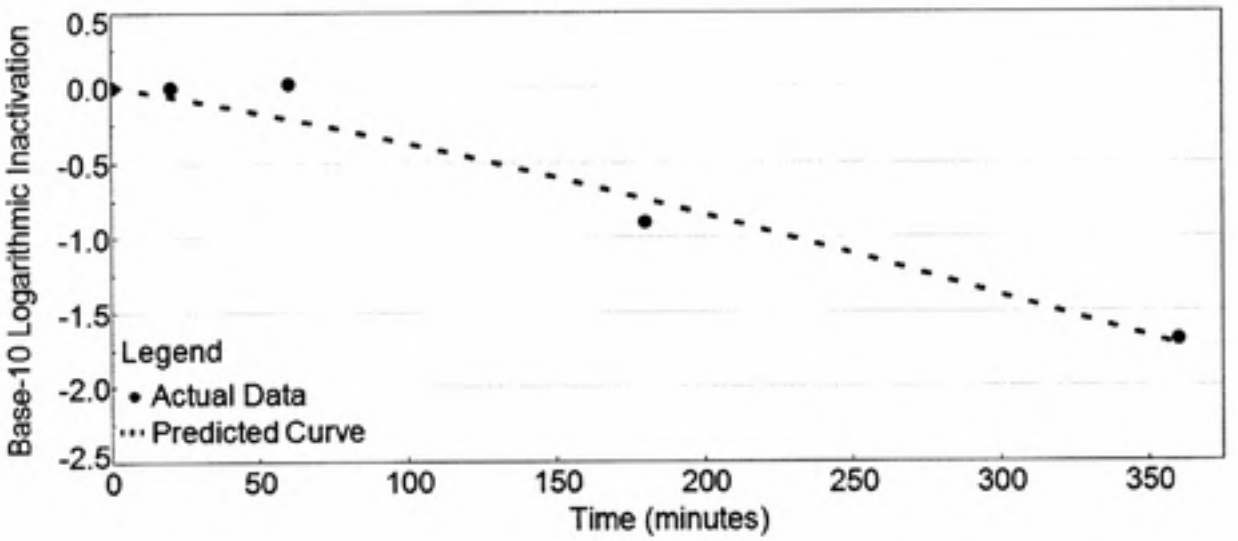


Figure 7: Shouldering Curve Fit by the Hom Model of the Disinfection of *Bacillus cereus* Spores in NOM-Negative Waters by 2 mg/L Metal Additive Dose

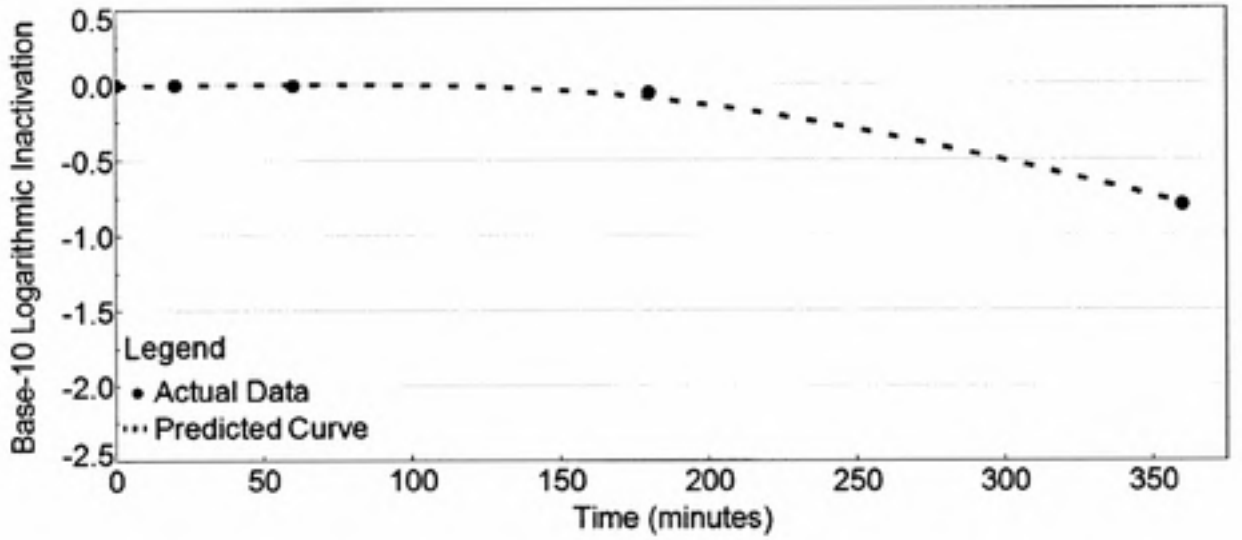


Figure 8: Shouldering Curve Fit by the Hom Model of the Disinfection of *Escherichia coli* in NOM-Negative Waters by 0.2 mg/L Metal Additive Dose

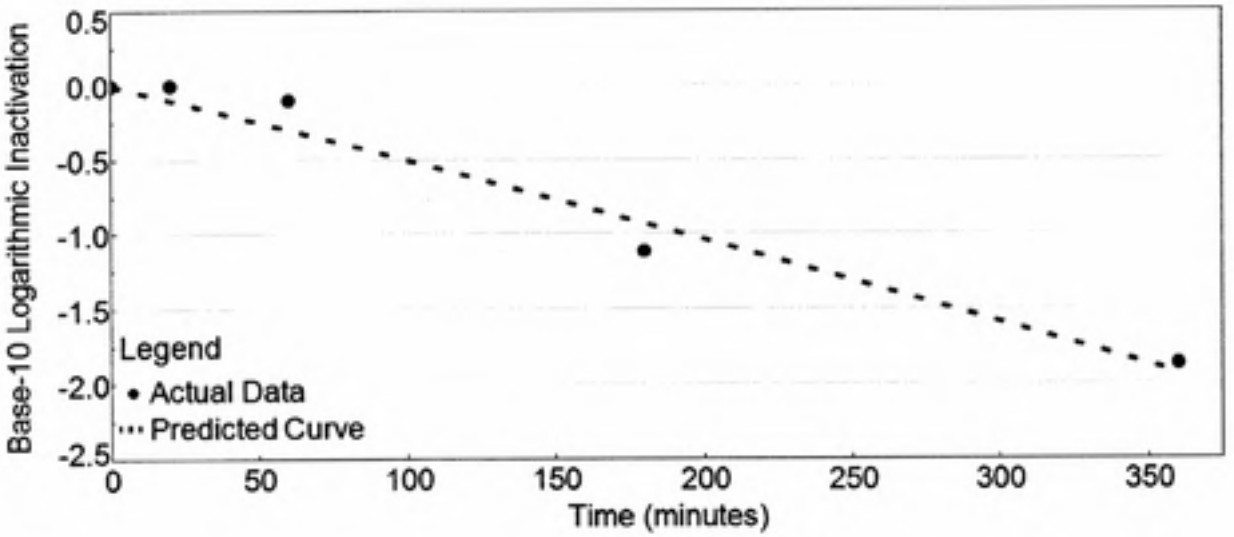


Figure 9: Shouldering Curve Fit by the Hom Model of the Disinfection of *Escherichia coli* in NOM-Negative Waters by 2 mg/L Metal Additive Dose

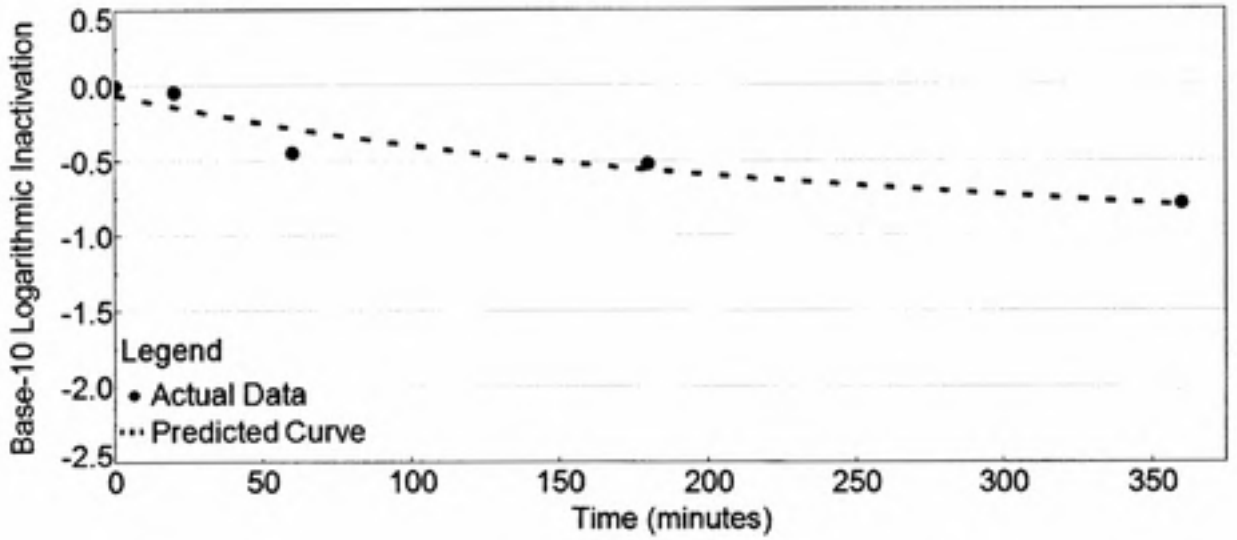


Figure 10: Tailing Curve Fit by the Hom Model of the Disinfection of MS2 Coliphage in NOM-Negative Waters by a 2 mg/L Metal Additive Dose

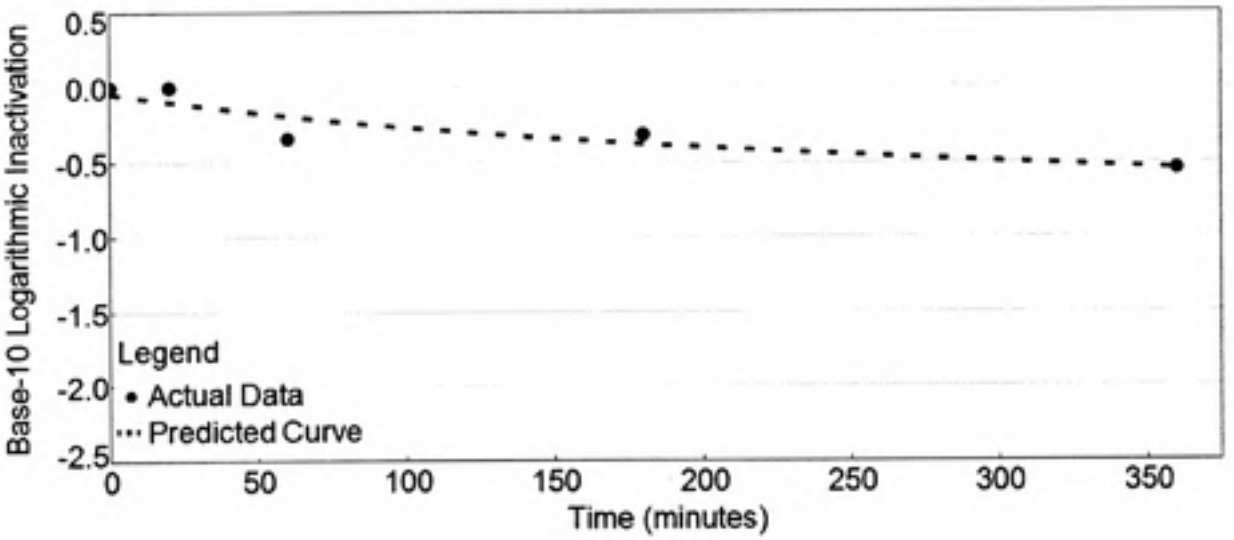


Figure 11: Tailing Curve Fit by the Hom Model of the Disinfection of *Bacillus cereus* Spores in NOM-Positive Waters by a 0.2 mg/L Metal Additive Dose

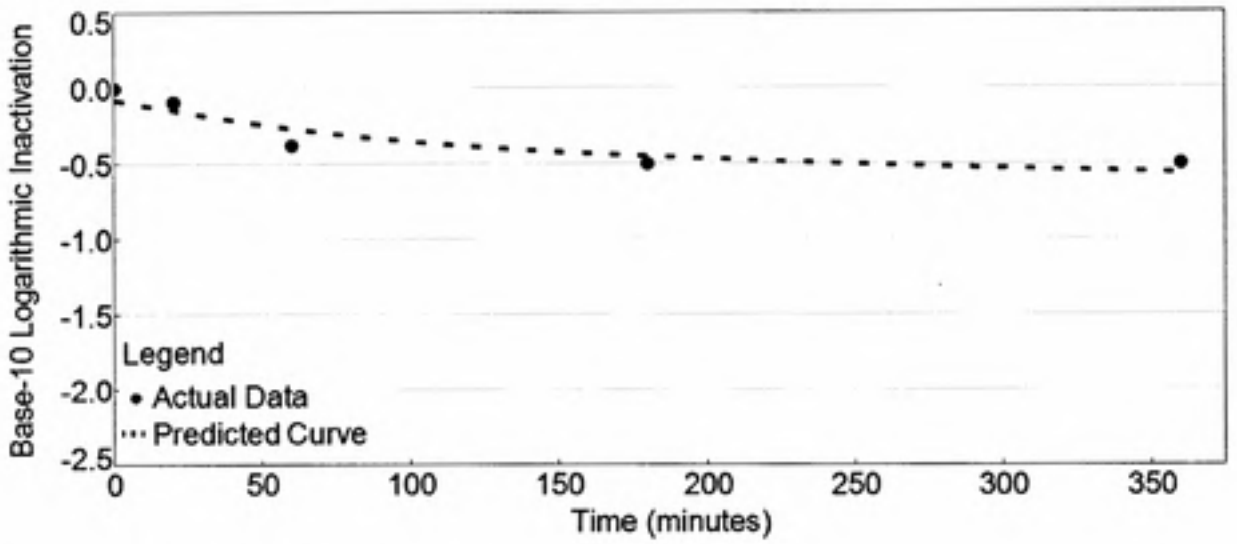


Figure 12: One-Hit Two-Population Curve Fit of the Disinfection of *Bacillus cereus* Spores in NOM-Positive Waters by a 2 mg/L Metal Additive Dose

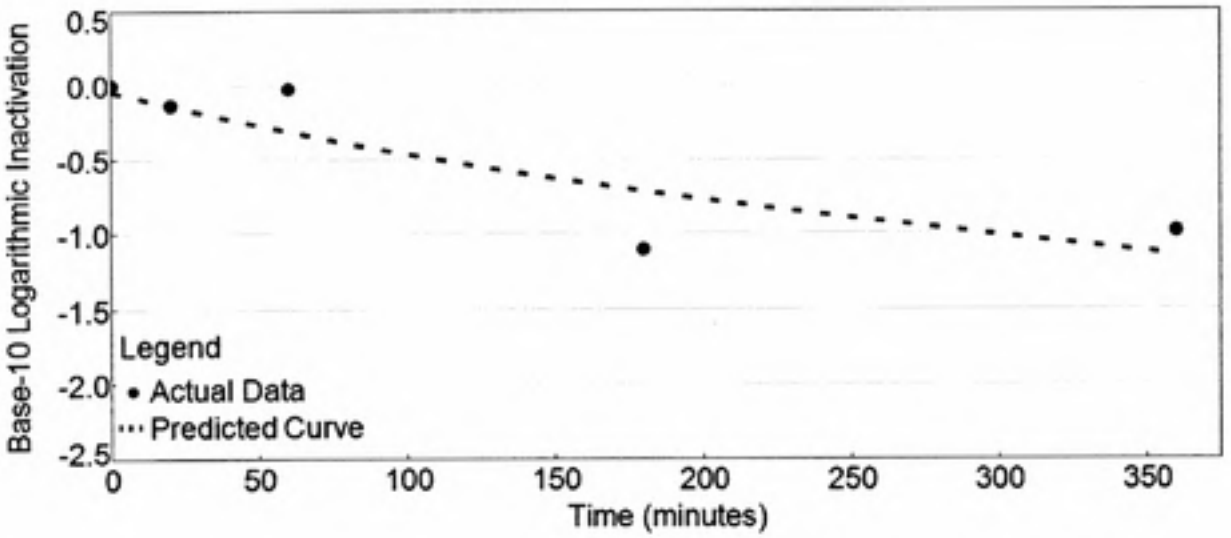


Figure 13: Tailing Curve Fit by the Hom Model of the Disinfection of *Escherichia coli* in NOM-Positive Waters by a 0.2 mg/L Metal Additive Dose

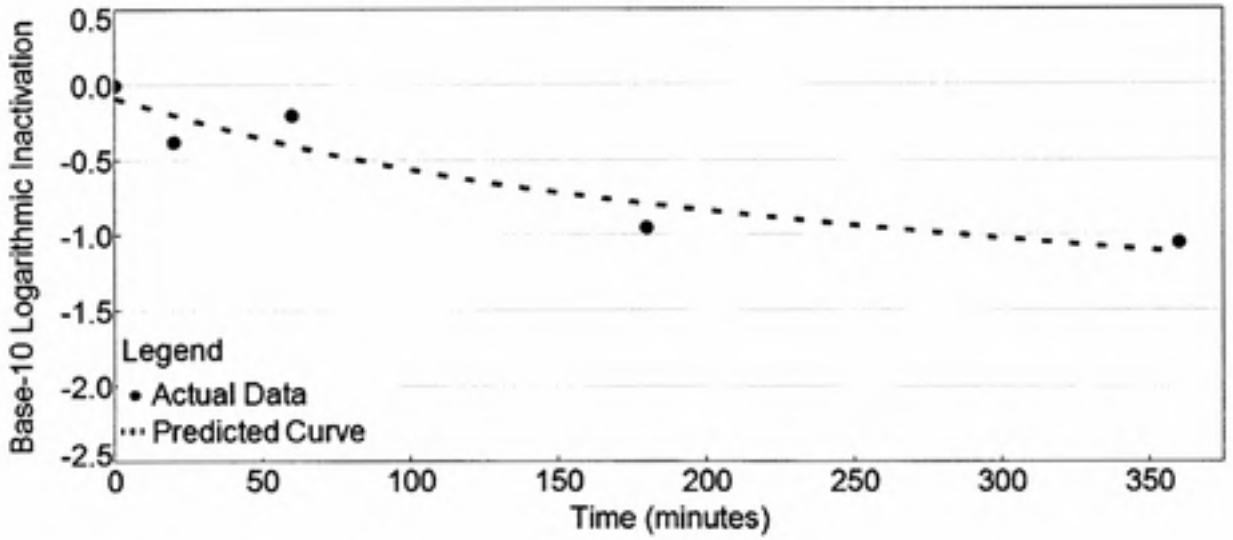


Figure 14: Tailing Curve Fit by the Hom Model of the Disinfection of *Escherichia coli* in NOM-Positive Waters by a 2 mg/L Metal Additive Dose

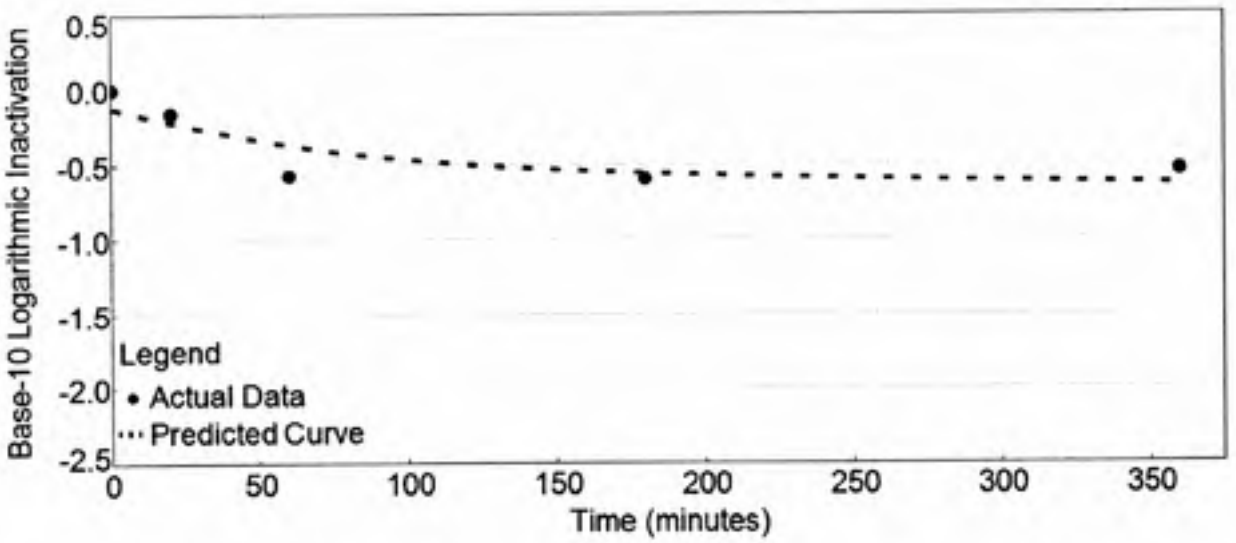


Figure 15: One-Hit Two-Population Curve Fit of the Disinfection of MS2 Coliphage in NOM-Positive Waters by a 2 mg/L Metal Additive Dose

Experimental Conditions	Rate Constant (k)	Time Exponent (m)	Kinetics Characterisation
Synthetic Water // *B. cereus* // 0.2 mg per L	-2.55 * 10-5	1.76	Shouldering
Synthetic Water // *B. cereus* // 2 mg per L	-1.21*10-3	1.23	Slight Shouldering
Synthetic Water // *E. coli* // 0.2 mg per L	-9.29*10-11	3.89	Shouldering
Synthetic Water // *E. coli* // 2 mg per L	-3.77*10-3	1.06	Slight Shouldering
Synthetic Water // MS2 // 2 mg per L	-3.55*10-2	0.526	Tailing
Organic Water // *B. cereus* // 0.2 mg per L	-2.25*10-2	0.539	Tailing
Organic Water // *E. coli* // 0.2 mg per L	-1.96*10-2	0.690	Tailing
Organic Water // *E. coli* // 2 mg per L	-5.30*10-2	0.516	Tailing

Table 6: Hom Model Parameters and Associated Kinetics Characterisation under Various Experimental Conditions for Microbe Disinfection by Metal Oxide Nanoparticles

Experimental Conditions	Affected Fraction (f)	Affected Fraction Decay Rate (k1)	Residual Fraction
Organic Water // *B. cereus* // 2 mg per L	69.9%	-2.27*10-2	30.1%
Organic Water // MS2// 2 mg per L	74.9%	-3.32*10-2	25.1%

Table 7: One-Hit Two-Population Model Parameters and Associated Residual Fractions under Various Experimental Conditions for Microbe Disinfection Kinetics by Metal Oxide Nanoparticles

Based on these results for the two-population kinetic modelling of the $\log_{10}$ reduction data, the susceptible populations follow first-order Chick-Watson disinfection kinetics, while the resistant population remains unchanged in concentration over time. For this reason, extrapolation analysis must be performed using the Hom model, as the One-Hit Two Population Model failed to converge in some cases. Fit values for the Hom model are reported in Table 5, all of which range from 89% to 100%, indicating it as a suitable means of extrapolation.

Evaluation of Disinfectant Concentration at 0 and 360 Minutes of Contact Time

Inductively-coupled plasma mass spectroscopy was performed, as described above in Methods and Materials, at 0 and 360 minutes of contact time. For each trial, triplicate measurements were taken, and averaged, and the percent root standard deviation calculated. Trials were done in triplicate themselves; the mean of measurements for each trial was therefore used to provide the trial-averaged data presented below in Tables 8-11. The full details of ICP-MS data are presented in the appendix. The data for NOM-negative water (Tables 8-9) and NOM-amended water (Tables 10-11) are presented separately. All numbers are reported to 3 significant figures.

ICP-MS Sample	Dose Eq. (ppb)	[$DISS_{Cu}$] (ppb)	Margin of Error (ppb)	[$DISS_{Zn}$] (ppb)	Margin of Error (ppb)
0.2 mg/L with Organisms	200.	108	13.4	168	21.3
2 mg/L with Organisms	2000	873	51.8	1360	23.2
0.2 mg/L without Organisms	200.	128	0.81	116	6.86
2 mg/L without Organisms	2000	1262	21.0	1100	37.2

Table 8: ICP-MS Trial-Averaged Concentrations of Dissolved Copper and Zinc Collected at 0 Minutes of Disinfectant Contact Time in NOM-Negative Buffered Water

ICP-MS Sample	Dose Eq. (ppb)	[$DISS_{Cu}$] (ppb)	Margin of Error (ppb)	[$DISS_{Zn}$] (ppb)	Margin of Error (ppb)
0.2 mg/L with Organisms	200.	98.3	1.48	137	39.1
2 mg/L with Organisms	2000	836	15.3	1920	197
0.2 mg/L without Organisms	200.	185	0.56	97.7	7.55
2 mg/L without Organisms	2000	1380	77.7	1160	43.5

Table 9: ICP-MS Trial-Averaged Concentrations of Dissolved Copper and Zinc Collected at 360 Minutes of Disinfectant Contact Time in NOM-Negative Buffered Water

As shown above in Tables 8 and 9, measured concentrations of dissolved copper and zinc in NOM-negative buffered water were always lower than added dose equivalents in every case. In NOM-negative buffered water with added microbes, between 43 and 96% of added dose equivalents were converted to measurable free metal forms. Reasons for measured metals concentrations being lower than doses were not investigated in this study. It is the possible that some amount of added metal oxide nanoparticles formed aggregates or other complexes that were lost during the process

of centrifugation, that some were internalised by microorganisms, or that some were physically lost from the water, perhaps by adsorption to the walls of pipets and sample containers.

ICP-MS Sample	Dose Eq. (ppb)	[$DISS_{Cu}$] (ppb)	Margin of Error (ppb)	[$DISS_{Zn}$] (ppb)	Margin of Error (ppb)
0.2 mg/L with Organisms	200.	121	13.0	286	6.20
2 mg/L with Organisms	$2.00*10^3$	411	62.0	1170	16.8
0.2 mg/L without Organisms	200.	121	54.2	330	14.5
2 mg/L without Organisms	$2.00*10^3$	445	51.4	1190	35.7

Table 10: ICP-MS Trial-Averaged Concentrations of Dissolved Copper and Zinc Collected at 0 Minutes of Disinfectant Contact Time in NOM-Amended Buffered Water

ICP-MS Sample	Dose Eq. (ppb)	[$DISS_{Cu}$] (ppb)	Margin of Error (ppb)	[$DISS_{Zn}$] (ppb)	Margin of Error (ppb)
0.2 mg/L with Organisms	200.	137	11.8	329	22.6
2 mg/L with Organisms	$2.00*10^3$	445	75.2	1160	43.5
0.2 mg/L without Organisms	200.	47.7	68.3	302	61.2
2 mg/L without Organisms	$2.00*10^3$	450	71.5	1510	103

Table 11: ICP-MS Trial-Averaged Concentrations of Dissolved Copper and Zinc Collected at 360 Minutes of Disinfectant Contact Time in NOM-Amended Buffered Water

As can be seen above in Tables 10 and 11, measured concentrations of dissolved copper and zinc were often lower than the added dose equivalent in most cases for NOM-amended buffered water as well. For copper, measured concentrations in NOM-amended water containing microbes range from 20-22% at the high metal dose to 60-69% at the low metal dose. For zinc in NOM-amended water containing microbes, measured concentrations were higher than added dose equivalents by as much as 65% at the lower target dose of 0.2 mg/L but they were lower by as much as 42% at the higher target dose of 2 mg/L. Again, the reasons for these differences in observed metals concentrations compared to doses were not specifically investigated. However, it is possible that

some amount of added metal oxide nanoparticles was lost to container walls or formed complexes or aggregates that were removed upon centrifugation.

In microbe-positive samples, it is possible that a percentage of the added metal oxide nanoparticles were complexed with or internalised by the test microorganisms, as was found by several studies in the literature review of this technical report (e.g. Brayner et al, 2006). Where the observed zinc concentration was higher than the dose added, it is possible that there was mechanical error in the measurement of the dissolved concentration of zinc. Another possibility is an error in metal oxide nanoparticle dosing. If the stock solution of zinc oxide nanoparticles was not sufficiently mono-dispersed, it is possible that the volume added to the test water contained more zinc oxide nanoparticles than calculated. This would lead to an under-reporting of the added dose equivalent, and could explain the increase in measured concentration of dissolved zinc.

Another noteworthy point is the substantially lower free copper concentration in NOM-amended water, as compared to NOM-negative water, at the 2 mg/L dose. This magnitude of lower measured concentration compared to dose was not observed at the lower dose of 0.2 mg/L., and did not appear to be present in the production of free zinc. In the presence of NOM, there was an approximate 55% decrease in free copper (411 and 445 ppb in NOM-amended waters at 0 and 360 minutes, respectively, versus 873 and 1262 ppb in NOM-negative waters at the same time points, for example). There are insufficient data to conduct a robust statistical evaluation of significance with regards to measured free copper concentrations relative to microbial reduction performance. However, it appears that the potential for NOM to suppress free copper presence or cause copper losses may have an impact on the ability of copper oxide nanoparticles to disinfect drinking water.

ICP-MS Sample	Dose Equivalents (ppb)	$\Delta DISS_{Cu}$ (ppb, from 0 to 360 Minutes)	$\Delta DISS_{Cu}$ Margin of Error (ppb)	$\Delta DISS_{Zn}$ (ppb, from 0 to 360 Minutes)	$\Delta DISS_{Zn}$ Margin of Error (ppb)
0.2 mg/L with Organisms	200.	-9.33	16.47	-31.3	63.9
2 mg/L with Organisms	$2.00*10^3$	-37.33	70.7	553	172
0.2 mg/L without Organisms	200.	57.33	1.37	-18.0	15.0
2 mg/L without Organisms	$2.00*10^3$	117.33	106.63	60.0	82.2

Table 12: ICP-MS Trial-Averaged Differences of Data Collected at 0 Minutes and 360 Minutes of Disinfectant Contact Time in NOM-Negative Buffered Water

ICP-MS Sample	Dose Equivalents (ppb)	$\Delta DISS_{Cu}$ 95% CI Lower Bound	$\Delta DISS_{Cu}$ 95% CI Upper Bound	$\Delta DISS_{Zn}$ 95% CI Lower Bound	$\Delta DISS_{Zn}$ 95% CI Upper Bound
0.2 mg/L with Organisms	200.	-25.8	7.13	-95.2	32.5
2 mg/L with Organisms	$2.00*10^3$	-108	33.4	381	725
0.2 mg/L without Organisms	200.	56.0	58.7	-33.0	-3.01
2 mg/L without Organisms	$2.00*10^3$	10.7	224	-22.3	142

Table 13: 95% Confidence Interval for ICP-MS Trial-Averaged Differences of Data Collected at 0 Minutes and 360 Minutes of Disinfectant Contact Time in NOM-Negative Buffered Water

ICP-MS Sample	Dose Equivalents (ppb)	Difference in Measured $DISS_{Cu}$ (ppb)	$DISS_{Cu}$ Margin of Error (ppb)	Measured $DISS_{Zn}$ (ppb)	$DISS_{Zn}$ Margin of Error
0.2 mg/L with Organisms	200.	15.7	50.5	42.3	30.9
2 mg/L with Organisms	$2.00*10^3$	34	113	-17.3	61.8
0.2 mg/L without Organisms	200.	-73.7	71.1	-28.7	65.0
2 mg/L without Organisms	$2.00*10^3$	4.33	171	324	141

Table 14: ICP-MS Trial-Averaged Differences of Data Collected at 0 Minutes and 360 Minutes of Disinfectant Contact Time in NOM-Amended Buffered Water

ICP-MS Sample	Dose Equivalents (ppb)	$\Delta DISS_{Cu}$ 95% CI Lower Bound	$\Delta DISS_{Cu}$ 95% CI Upper Bound	$\Delta DISS_{Zn}$ 95% CI Lower Bound	$\Delta DISS_{Zn}$ 95% CI Upper Bound
0.2 mg/L with Organisms	200.	-34.8	66.1	11.4	73.3
2 mg/L with Organisms	$2.00*10^3$	-79.3	147	-79.2	44.5
0.2 mg/L without Organisms	200.	-145	-2.53	-93.7	36.4
2 mg/L without Organisms	$2.00*10^3$	-167	176	183	466

Table 15: 95% Confidence Interval for ICP-MS Trial-Averaged Differences of Data Collected at 0 Minutes and 360 Minutes of Disinfectant Contact Time in NOM-Amended Buffered Water

In NOM-negative buffered water, dosed with either the 0.2 mg/L or the 2 mg/L concentration of metal oxide nanoparticles, there were no significant differences in concentrations of dissolved copper in organism-positive samples taken at 0 and 360 minutes (the margins of error of both measurements included 0). In the same type of water, at the 0.2 mg/L dose of metal oxide nanoparticles, measured concentrations of dissolved zinc also were not significantly different between samples taken at time t=0 and t=360 minutes (the margin of error of the measurement included 0). However, for zinc oxide nanoparticles at the 2 mg/L dose in organism-positive samples, there was a statistically significant difference, specifically an increase, observed in concentrations of dissolved zinc between measurements taken at 0 minutes and 360 minutes of contact time (the 95% CI for the value of the difference is between 381 and 725 ppb).

In NOM-amended buffered water containing organisms and dosed with 2 mg/L of metal oxide nanoparticles, there were no significant differences in concentrations of copper or zinc between the 0 and 360 minute samples (the margins of error of both measurements included 0). Likewise, in the same water dosed with 0.2 mg/L of metal oxide nanoparticles there were no significant differences

in concentrations of dissolved copper between samples taken at 0 and 360 minutes of contact time (the margin of error of the measurement included 0). However, for zinc oxide nanoparticles at the 0.2 mg/L dose in organism-positive samples, there was a statistically significant difference, specifically an increase, observed in concentrations of dissolved zinc between measurements taken at 0 minutes and 360 minutes of contact time (the 95% CI for the value of the difference is between 11.4 and 73.3 ppb).

Extrapolation to Meet Specified WHO Log_{10} Reduction Performance Targets

The amount of time required, according to the data presented here, to reach the aforementioned performance targets of the World Health Organisation for household water treatments can be extrapolated from the respective model's parametric data. For each of the twelve experimental conditions, the performance target, and time required to reach the performance target are summarised in Table 8 below. Those data sets that were best fit by the One-Hit Two-Population Model, but for which the Hom model was nevertheless used for extrapolation, are bolded in Table 8 below. For these data sets, such extrapolation is presented for illustrative purposes only.

The term "modular" in "modular hours" below refers to a method of rounding in which any fragment of a whole number is presumed to round up to the nearest higher whole number. For example, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, and 1.9 would all be rounded to 2. This method of rounding is used frequently in demographic analysis, econometrics, and other related fields, in order to provide a conservative estimate of consumed resources or estimated costs of a particular action, good, or service. This method of rounding was used here to provide a conservative estimate of the time required to reach a particular level of microbial disinfection.

Experimental Conditions	Model Used	"Protective" Performance Target (Number of Log_{10} Reductions)	Time Required (Minutes)	Time Required (Modular Hours)
Synthetic Water // *B. cereus* // 0.2 mg per L	Hom	2	604	11
Synthetic Water // *B. cereus* // 2 mg per L	Hom	2	414	7
Synthetic Water // *E. coli* // 0.2 mg per L	Hom	2	453	8
Synthetic Water // *E. coli* // 2 mg per L`	Hom	2	372	7
Synthetic Water // MS2// 0.2 mg per L	Not Applicable	3	Not Applicable	Not Applicable
Synthetic Water // MS2 //2 mg per L	Hom	3	4606	77
Organic Water // *B. cereus* // 0.2 mg per L	Hom	2	4127	68
Organic Water // *B. cereus* // 2 mg per L	**Hom**	**2**	**2.11*1028**	**3.52*1026**
Organic Water // *E. coli* // 0.2 mg per L	Hom	2	815	14
Organic Water // *E. coli* // 2 mg per L	Hom	2	1137	19
Organic Water // MS2// 0.2 mg per L	Not Applicable	3	Not Applicable	Not Applicable
Organic Water // MS2 //2 mg per L	**Hom**	**3**	**2.62*105**	**4.38*103**

Table 16: Time Required to Reach W.H.O. "Protective" Performance Targets under Various Experimental Conditions

Mixed-Model Analysis

Once all modelling and extrapolations were performed, analysis was performed in order to ascertain: (1) the effect of metal dose on estimated disinfection efficacy within a particular aqueous matrix, and (2) the effect of dissolved organic matter in the buffered test water on estimated disinfection efficacy within a particular dose range, as described above. For the first, on the effect of dose, baseline parametric analysis was performed; specifically, the disinfection efficacy of the 0.2 mg/L dose was compared with the disinfection efficacy of the 2 mg/L dose across time. Disinfection of MS2 bacteriophage was not subjected to this analysis, since the 0.2 mg/L dose yielded no

significant disinfection results while the 2 mg/L dose did. The doses yielded significantly different disinfection efficacy by definition. The results of this analysis are presented below. The term "amplifies" refers to a positive dose-response relationship, with increasing dose resulting in increasing inactivation efficacy. Increased dose resulted in increased disinfection of *B. cereus* spores and *E. coli* in synthetic test water but did not increase their disinfection efficacy in organic water.

Experimental Conditions	**Estimate of β**	**95% CI**		**Statistically Significant?**	**Dose Effect**
Synthetic Water // *B. cereus*	-0.00263	-0.00342	-0.00184	Yes	Amplifies
Synthetic Water // *E. coli*	-0.00351	-0.00423	-0.00280	Yes	Amplifies
Synthetic Water // MS2	Not Applicable	Not Applicable		Not Applicable	Not Applicable
Organic Water // *B. cereus*	-0.000124	-0.000984	0.000737	No	None
Organic Water // *E. coli*	-0.0000710	-0.00125	0.00111	No	None
Organic Water // MS2	Not Applicable	Not Applicable		Not Applicable	Not Applicable

Table 17: Baseline Parametric Analysis Results for Statistical Significance between Metal Additive Doses for Test Microbe Disinfection at Various Experimental Conditions of Test Water

To ascertain the effect of the presence of dissolved organic matter obtained from Catfish Lake in test water on the microbial disinfection efficacy of the two doses of the metal additive, similar analysis was performed for *B. cereus* and *E. coli*. For MS2 coliphage, analysis of parametric joint confidence spread was performed. The results are presented below in Tables 10 and 11. Note that the comparison between the 0.2 mg/L dose for MS2 coliphage yields a statistically insignificant result by definition, since no significant disinfection was observed in either test water. As can be seen in Table 10, the disinfection of *B. cereus* at the 0.2 mg/L dose is not significantly altered by the addition of NOM. The disinfection of *E. coli* in contrast, displayed a slight amplification effect with the addition of NOM (that is, the addition of NOM appeared to increase the disinfection efficacy of

the metal nanoparticle dose). For disinfection of both *B. cereus* and *E. coli* at the 2 mg/L dose of metal oxide nanoparticle, the addition of NOM was shown to retard disinfection efficacy. No significant effect of the addition of NOM was found for the inactivation of MS2 coliphage by the 2 mg/L metal oxide nanoparticle dose in either NOM-negative or NOM-amended waters.

Experimental Conditions	Estimate of β	95% CI		Statistically Significant?	NOM Effect
B. cereus // 0.2 mg per L	-0.000345	-0.000949	0.000259	No	None
E. coli// 0.2 mg per L	-0.00157	-0.00296	-0.000173	Yes	Amplifies
MS2 // 0.2 mg per L	Not Applicable	Not Applicable		Not Applicable	Not Applicable
B. cereus // 2 mg per L	0.00285	0.00199	0.00371	Yes	Retards
E. coli // 2 mg per L	0.00188	0.000698	0.00305	Yes	Retards

Table 18: Chick-Watson Baseline Parametric Analysis Results for Statistical Significance between Test Waters for Various Experimental Conditions of Metal Additive Dose and Test Microbe Disinfection

Conditions for MS2 Coliphage	Estimate of k	95% CI		Estimate of m	95% CI	
Synthetic Water // 2 mg per L	-0.0355	-0.0963	0.0252	0.526	0.215	0.838
Organic Water // 2 mg per L	-0.152	-0.394	0.0892	0.239	-0.0688	0.547
Overlap?	Yes			Yes		
Significant?	No			No		
NOM Effect	None					

Table 19: Hom-Model Baseline Parametric Analysis Results for Statistical Significance between Test Waters for Various Experimental Conditions of Metal Additive Dose and Test Microbe Disinfection (Chick-Watson and Hom Comparisons Shown Separately)

Effect of Metal Nanoparticle Dose on *E. coli*, *B. cereus*, and MS2 Coliphage Disinfection for Buffered Water (Presence of Dissolved Organic Matter Held Constant for Comparisons)

Results for $\log_{10}$ reductions of test microbes in 6 hours by the two doses of metal oxide nanoparticles in both test waters are summarized in Table 11. Disinfection of *Bacillus cereus* and of *Escherichia coli* in buffered water that lacks dissolved organic matter was found to exhibit a significant dose-response relationship. For both microbes, the increase of dose from 0.2 milligrams per litre to 2 milligrams per litre was found to increase disinfection (i.e. to have lowered the survival ratio) over the 6 hour course of time.

Disinfection of *B. cereus* and *E. coli* in waters with dissolved organic matter on the other hand was not found to exhibit a significant dose-response relationship, as the extent of disinfection, measured as $\log_{10}$ reductions of the survival ratio of about 0.5 for *B. cereus* spores and 1.0 for *E. coli*, were statistically similar for both doses of metal nanoparticles. Disinfection of MS2 displayed a dose-response relationship by definition, since there was no significant reduction at a 0.2 mg/L dose, but a measured reduced of approximately a 0.5 $\log_{10}$ reduction at a 2 mg/L dose. Overall, the presence of dissolved organic matter in the test water resulted in less disinfection of some microbes, such as *B. cereus* at both doses of metal NPs and *E. coli* at both doses of metal NPs.

Experimental Conditions	0.2 mg/L Dose	2 mg/L Dose
Synthetic Water // *B. cereus*	0.79	1.69
Synthetic Water // *E. coli*	0.80	1.87
Synthetic Water // MS2	Not significantly different from 0	0.78
Organic Water // *B. cereus*	0.54	0.49
Organic Water // *E. coli*	0.98	1.05
Organic Water // MS2	Not significantly different from 0	0.53

Table 20: Experimental Conditions of Test Microbe, Test Water and Metal Dose and Resultant $\log_{10}$ Reductions of Culturable Microbes after 6 Hours

Effect of the Presence of Dissolved Organic Matter on the Efficacy of the Metal Additive Doses and Their Disinfection of *B. cereus*, *E. coli*, and MS2 Coliphage (Amount of Dose Held Constant for Comparisons)

The 0.2 milligram per litre dose of the metal additives did not produce statistically significantly different disinfection of *B. cereus* between waters with and without NOM, with $\log_{10}$ reductions of 0.54 and 0.79 respectively. This lack of statistical significance is due to the breadth of the margin of error; more variability was present in replicate trial measurements of *B.* cereus disinfection leading to an overlap in the margins-of-error of the NOM-negative and NOM-amended disinfection curves. It is noteworthy however that the *B. cereus* $\log_{10}$ reduction was less in water amended with NOM as compared to NOM-negative buffered water, suggesting an inhibitory effect associated with NOM on *B. cereus* spore disinfection.. The metal additives produced significantly different disinfection of *E. coli* between waters with and without NOM, with the 0.2 mg/L metal additives dose resulting in $\log_{10}$ reductions of 0.98 and 0.8 in water with and without NOM, respectively. Hence for *E. coli*, disinfection by 0.2 mg/L of metal oxide nanoparticles was greater in water with NOM than without NOM, suggesting a potential enhancement effect associated with NOM presence.

Though the gap in mean disinfection efficacy is smaller than that for *B.* cereus at the same dose, less variability in replicate trial measurements resulted in a smaller margin-of-error for disinfection curves for both NOM-negative and NOM-amended water. At the 0.2 mg/L dose, the presence of NOM was found to amplify the effect of the metal additives against *E. coli*, possibly implying that at the concentration studied (approximately 10 mg/L), NOM can increase the concentration of bioactive forms of copper and zinc oxide nanoparticles against bacteria in test waters similar to those used here, or that in small amounts, NOM can somehow make microbial populations more susceptible to the disinfectant.

The 2 milligram per litre dose of the metal additives did not produce significantly different inactivation of MS2 coliphage between waters with and without NOM. However, for *B. cereus* and *E. coli*, the 2 mg/L dose of metal oxide nanoparticles resulted in significant differences in $\log_{10}$ reductions in test waters with and without NOM (*E. coli*: 1.05 versus 1.87; *B. cereus* spores: 0.49 versus 1.69). In these cases, the presence of NOM was found to have a negative effect on the disinfection efficacy of the metal additives against the bacteria tested. The reasons for these differences were not specifically investigated. However, it is possible that when the metal additives are present in an amount like the tested dose of 2 mg/L, NOM can decrease the bioactive forms of copper and zinc oxide nanoparticles, or perhaps protect the test microbes against them, in the type of test water used here.

XI. Discussion

Estimated Efficacy of Copper and Zinc Oxide Nanoparticles as *B. cereus*, *E. coli*, and MS2 Coliphage Disinfectants in Water (Relative to WHO Disinfection Performance Targets)

In waters without dissolved organic matter, the 0.2 milligrams per litre dose of copper and zinc oxide nanoparticles would have taken 11 hours and 8 hours to reach the W.H.O. minimum "protective" performance target of 2 $\log_{10}$ (99%) reduction for both disinfection of bacteria and protozoan parasites (represented here by *E. coli* and *B. cereus*, respectively) by household water treatment systems (HWTS). No significant disinfection at this dose was observed for MS2 coliphage. The 2 milligrams per litre dose, on the other hand, would have taken 7 hours to reach the W.H.O.'s minimum "protective" performance target of 2 $\log_{10}$ (99%)reduction for both bacteria and protozoan parasites, and 77 hours to reach the W.H.O.'s minimum target of 3 $\log_{10}$(99.9%) reduction of MS2 coliphage, based on the kinetic modelling techniques previously described above.

In relatively solids-free (low-turbidity) waters, therefore, we can conclude that copper and zinc oxide nanoparticles, as used here in both tested doses, would provide reasonable same-day point-of-use household (POUH) drinking-water treatment against bacteria. For viruses however, disinfection with these metals would have to be combined with other means of disinfection to control enteric viruses as modelled by MS2. In addition, the observation that both the bacteria and protozoan surrogate "protective" performance target can be achieved in a reasonable contact time, indicating that the "protective" level of performance is achievable for two of the three classes of test microbes, makes it possible for disinfection with copper and zinc metal oxide nanoparticles to potentially meet the W.H.O. "interim" performance target. These results, though not as broadly applicable to all three classes of pathogens as originally desired, reasonably align with the original experimental objectives. Before such an "interim" performance target could be met, epidemiological field studies would be required to determine if the use of these metal nanoparticle additives in

drinking water produced a significant reduction in diarrhoeal disease risk in consumers of treated waters, compared to a control group. With supporting health data, the future use of these nanoparticles as one part of an overall treatment and management strategy may be feasible as an alternative POUH water treatment technology for low-resource settings.

For waters containing dissolved organic matter, the low dose of the copper and zinc oxide nanoparticle additive would have required approximately 14 hours to reach the W.H.O. "protective" performance target for *E. coli* disinfection and 68 hours to reach the W.H.O. "protective" performance target for *B. cereus* disinfection. No significant difference was observed for disinfection of MS2 coliphage. Therefore, disinfection of such NOM-amended water by the 0.2 mg/L metal oxide nanoparticle dose alone would not achieve adequate disinfection to merit the protective level of performance in a practical period of time.

The high (2 mg/L) dose of the metal oxide nanoparticle additive in NOM-amended water would have required several months to reach the W.H.O. "protective" performance target for *B. cereus* disinfection, 19 hours for *E. coli* disinfection, and several months for MS2 coliphage disinfection. Thus, disinfection of *E. coli* in NOM-amended water yielded results consistent with effective use as an overnight disinfectant. However, disinfection of *B. cereus* and MS2 coliphage, regardless of model applicability, would certainly take far longer than 18 hours to reach the W.H.O. "protective" performance targets of acceptable efficacy.

The differences in disinfection performance of copper and zinc metal oxide nanoparticles between waters with and without NOM indicates potentially limits applicability of this technology for POUH water treatment in settings with waters containing NOM. This implies that the metal additive used here may require use in combination with other treatment processes such as physical filtration

methods, or may require collected waters to be given time to settle out particulate matter and be decanted into separate vessels prior to addition of the metal nanoparticle additive for disinfection.

Other Factors Influencing Disinfection Efficacy

Chemical Reactivity of Delivered Nanoparticle Copper and Zinc

For reasons described previously above, a particularly stable form of uncharged copper and zinc nanoparticles was used, specifically the oxide form. Other forms of the metal nanoparticles with similar stability as stored compounds, but more reactive in the environment of actual use, may have resulted in different disinfection efficacy than observed with those metal oxide nanoparticles tested.

For example, uncharged nanoparticles of copper or zinc stabilised with some form of saccharide or amino acid may be feasible for use in the context of consumable drinking water disinfectants (Badireddy 2011, Sobsey 2011). Furthermore, uncharged nanoparticles of copper or zinc stabilised with nano-scale carbon structures (e.g., buckminsterfullerenes, or "buckyballs") or perhaps not stabilised at all, though not practical for consumable POUH water treatment, may find application in other contexts, such as high-dose reactive additives in fixed filtration systems, or treatment alternatives for wastewater, recycled grey water, or agricultural waters that are not intended for final human consumption.

Copper and Zinc Concentrations in Test Water and Potential Changes Over Time

As mentioned above, the measured concentrations of dissolved copper and zinc differed from the added dose equivalents to varying degrees. The per cent of the added dose equivalent that was measurable as free metal is presented below.

ICP-MS Sample	% [DISSCu], 0 Minutes	% [DISSCu], 360 Minutes	% [DISSZn], 0 Minutes	% [DISSZn], 360 Minutes
0.2 mg/L with Organisms	54	49.2	84.0	68.5
2 mg/L with Organisms	43.7	41.8	68.0	96.0
0.2 mg/L without Organisms	64.0	92.5	58.0	48.9
2 mg/L without Organisms	63.1	69.0	55.0	58.0

Table 21: ICP-MS Trial-Averaged Percentages of Added Dose Equivalent Conversion to Dissolved Metal—Data Collected at 0 Minutes and 360 Minutes of Disinfectant Contact Time in NOM-Negative Buffered Water

ICP-MS Sample	% [$DISS_{Cu}$], 0 Minutes	% [$DISS_{Cu}$], 360 Minutes	% [$DISS_{Zn}$], 0 Minutes	% [$DISS_{Zn}$], 360 Minutes
0.2 mg/L with Organisms	60.5	68.5	143	164.5
2 mg/L with Organisms	20.55	22.25	58.5	58
0.2 mg/L without Organisms	60.5	23.85	165	151
2 mg/L without Organisms	22.25	22.5	59.5	75.5

Table 22: ICP-MS Trial-Averaged Percentages of Added Dose Equivalent Conversion to Dissolved Metal—Data Collected at 0 Minutes and 360 Minutes of Disinfectant Contact Time in NOM-Amended Buffered Water

No significant changes in free copper concentrations were found between measurements taken initially, at time t = 0 minutes, and finally, at time t = 360 minutes for waters with and without dissolved organic matter added in organism-positive samples. Measured copper concentrations in both NOM-amended and NOM-negative waters at the 0.2 mg/L dose were approximately in the range of 0.1 to 0.15 mg/L, and at the 2 mg/L dose they were approximately in the range of 0.8 to 1.5 mg/L in samples taken at both 0 and 360 minutes. Levels of free zinc were observed to change between the start and the conclusion of the experiment; but observed changes were inconsistent.

The 0.2 mg/L dose of zinc oxide nanoparticles in NOM-amended water as well as the 2 mg/L dose of zinc oxide nanoparticles in NOM-negative water exhibited measurable increases in concentrations of dissolved zinc between samples taken at 0 and 360 minutes of contact time. For the 0.2 mg/L dose of zinc oxide nanoparticles in NOM-amended water, the approximate measured concentration of dissolved zinc at t=0 minutes was 0.286 mg/L, and at t =360 minutes was 0.329 mg/L. This measurable increase has a 95% CI between 11.4 and 73.3 parts per billion, or between 0.0114 and 0.0733 mg/L. For the 2 mg/L dose of zinc oxide nanoparticles in NOM-negative water, the approximate measured concentration of dissolved zinc at t=0 minutes was 1.36 mg/L, and at t =360 minutes was 1.92 mg/L. This measurable increase has a 95% CI between 381 and 725 parts per billion, or between 0.381 and 0.725 mg/L.

Therefore, possible differences in available disinfectant concentration over time as a factor in variations in disinfection kinetics of the test organisms were not relevant to copper, but may have been relevant to zinc. Due to time constraints and other limitations of this study, further sampling and characterisation of dissolved metal concentrations at contact times other than 0 and 360 minutes were not performed, impacting the ability to draw firm conclusions about the effects of dissolved metal concentration or changes in their concentrations on the test organisms *in situ*. For future research, if possible, such sampling and characterisation would be desirable.

Physical State of Test Microbes and Disinfectant

The effects of disinfection on test microbes may be influenced by their physical state. "Lone" or dispersed microbes (i.e. isolated bacterial cells/spores or isolated virions) present different, and possibly more accessible, targets for chemical disinfectants than "grouped" or aggregated microbes do. The physical state of the test microbes could not be determined by our experimental methods, nor was it possible to observe or account for their potential physical association with dissolved

organic matter in the test water, where applicable. However, the fit of the disinfection kinetics data to the Hom model and the One-Hit Two population model can possibly be explained in terms of aggregation. Any "shouldering", or lag in disinfection in the Hom model could be a reflection of initial presence of aggregates that subsequently disperse into the solution. Any "tailing" or tapering of disinfection in the Hom model could be a reflection of the formation or persistence of aggregates after a certain amount of time consistent with their initial presence at low concentrations.

For the One-Hit Two-Population model, the presence of an "unaffected" population throughout the duration of the experiment could be a reflection of a sustained population of microbial aggregates that neither increased nor decreased as the experiment went on. The same also applies to the disinfectant; preliminary laboratory experimentation indicated that the nanoparticle additives used here remained disaggregated for at least 360 minutes in test waters (Badireddy, 2011). The NOM-negative test water used with HEPES buffer was without other organic matter, and buffered to a typical natural water pH level. The water was also microbe-free, except for added test microbes. The extent to which the presence of microbes somehow induced physical association between the nanoparticles and other microbes, or the extent to which the presence of dissolved organic matter induced some physical association between nanoparticles themselves, were not specifically investigated in this study, and could have influenced variation in the microbial disinfection kinetics activity observed here.

Physiological state of E. coli *and* B. cereus *spores*

The *E. coli* cells used here were in log-phase, meaning that they were growing at their maximum rate and rapidly approaching the maximum culturable density per unit volume possible in the given growth medium at the time of use. Log-phase *E. coli* cells, especially when grown in high-density environments, as they were here, can exhibit markedly different physical and physiological

properties than cells sampled earlier in the growth curve, or than in stationary-phase *E. coli* (Sobsey, 2010). Potential presence of chains of attached cells with fused cell walls, giving multiples of cells physically bound together in free solution as can occur in log-phase cultures, may have behaved, in effect, similar to aggregates of microbes. Unlike simple aggregation of microbes, such chains of cells with fused cell walls would not have subsequently dissociated in significant numbers in still, un-agitated waters, unless cell division and separation processes continued to occur in test waters.

B. cereus spores exhibit measurable hydrophobic properties that could tend to promote aggregation in aqueous solutions (Husmark et al, 1992; Ronner et al, 1990, Hornstra et al, 2007; Tauveron et al, 2006). The stock spore solution was one of very high density and spore aggregates were therefore likely to form; mutual hydrophobic preferences may have preserved such spore aggregates when they were placed in the test waters used here (Sobsey, 2011). The potential presence of both these semi-permanent aggregates (fused chains of multiple *E. coli* log-phase cells and hydrophobically-associated *B. cereus* spore clusters) may explain the fit of the One-Hit Two-Population model to some disinfection kinetics data.

XII. Conclusions

The findings presented here indicate that copper and zinc oxide nanoparticles, in concentrations within the limits for consumable copper and zinc as designated by the U.S. FDA and the World Health Organisation, have the potential to serve as "interim" point-of-use household water treatments, under the definition of the W.H.O.'s "protective" performance benchmarks, if complemented with epidemiological data of human health impact. For the three classes of microorganisms, bacteria (*E. coli*), protozoan parasite (oo)cyst surrogates (*B. cereus* spores), and enteric viruses (MS2 coliphage), for which there are specific performance targets for such water treatment technologies, the metal additives studied here meet the specified $\log_{10}$ reduction performance targets for two out of the three categories, the bacteria, and the protozoan parasite surrogate, in a length of time appropriate for same-day or overnight usage, in waters without appreciable amounts of dissolved natural organic matter.

In NOM-negative waters, the 0.2 and 2 mg/L doses of the metal oxide nanoparticles achieved 0.79 and 1.69 $\log_{10}$ reductions, respectively, of *B. cereus* spores and 0.8 and 1.87 $\log_{10}$ reductions respectively, of *E. coli* after 6 hours. Based on these observed results and by extrapolation with appropriate disinfection kinetic models, the 0.2 and 2 mg/L doses of the metal oxide nanoparticles used here would have achieved the WHO protective performance level of 2 $\log_{10}$ reduction of *B. cereus* spores at approximately 11 and 7 hours respectively. Also by extrapolation from observed $\log_{10}$ reductions for *E.* coli log-phase cells at the 0.2 and 2 mg/L doses, the WHO protective level of performance of 2 $\log_{10}$ reduction would be achieved in about 8 and 7 hours, respectively.

However, in NOM-amended waters, the metal oxide nanoparticle additive did not yield sufficiently extensive and rapid disinfection of *B. cereus* spores and *E. coli* log-phase cells at the 0.2 and 2 mg/L dose to achieve the WHO recommended 2 $\log_{10}$ reduction. For *B. cereus* spores, 0.54 and 0.49 $\log_{10}$

reductions were achieved after 6 hours by copper and zinc oxide nanoparticle doses of 0.2 and 2 mg/l, respectively. Based on extrapolation from these data using an appropriate kinetic model, the WHO protective performance level of 2 $\log_{10}$ reductions of *B. cereus* spores would be achieved after approximately 68 and $3.52*10^{26}$ hours, for the 0.2 and 2 mg/L doses, respectively. For *E. coli* log-phase cells, 0.98 and 1.05 $\log_{10}$ reductions were achieved after 6 hours at copper and zinc oxide nanoparticle doses of 0.2 and 2 mg/L, respectively. Based on extrapolation from these data using an appropriate kinetic model, the WHO protective performance level of 2 $\log_{10}$ reduction of *E. coli* would be achieved after approximately 14 and 19 hours for the 0.2 and 2 mg/L doses, respectively.

For coliphage MS2 in both NOM-negative and NOM-amended waters, the 0.2 mg/L dose did not yield significant disinfection at all. At the 2 mg/L dose there was a 0.78 $\log_{10}$ reduction after 6 hours in NOM-negative waters and a 0.53 $\log_{10}$ reductions after 6 hours in NOM-amended waters. Disinfection performance did not meet the W.H.O. 3 $\log_{10}$ reduction performance target in a time frame consistent with overnight use for all copper and zinc oxide nanoparticle doses and both water qualities tested. At the 2 mg/L dose, reaching the WHO protective performance level of 3 $\log_{10}$ reductions would require approximately 77 hours in NOM-negative water and approximately 438,000 hours in NOM-amended water.

A dose-response effect for the metal oxide nanoparticle additive was found for disinfection of *B. cereus* spores and *E. coli* log-phase cells in NOM-negative waters (at the alpha level of 0.05), where a higher dose corresponded with a higher degree of disinfection. By default, a dose-response relationship was also found for disinfection of MS2 coliphage in NOM-negative waters, since the 0.2 mg/L dose yielded no significant disinfection, whereas the 2 mg/L dose did. In contrast, no dose-response relationship (at the alpha level of 0.05) was found for NOM-amended waters for disinfection of *B. cereus* spores or *E. coli* log-phase cells. For MS2 coliphage virions in NOM-

amended water, a dose-response effect was observed by default, since (similarly to the case of disinfection of MS2 coliphage in NOM-negative water) the 0.2 mg/L dose yielded no significant disinfection, whereas the 2 mg/L dose did.

The research presented here indicates that for low-turbidity waters also low in natural organic matter, including water allowed to sit for enough time to allow particulates to settle and then be further decanted, treatment at the point-of-use by copper and zinc oxide nanoparticles at the doses studied here can fulfil the W.H.O. protective performance targets. By disinfecting bacteria and protozoan parasite (oo)cyst surrogates to a sufficient degree (2 $\log_{10}$ reductions) within an amount of time consistent with same-day or overnight use in NOM-negative waters, both the 0.2 and 2 mg/L metal oxide nanoparticle doses would have met the W.H.O. protective performance targets in an amount of time consistent with same-day or overnight use. Also, if used in combination with other treatment technologies to further increase enteric virus reduction, the higher copper and zinc metal oxide nanoparticle dose of 2 mg/L may be a potentially effective and feasible treatment to meet the WHO protective performance target of 3 $\log_{10}$ reductions for enteric virus surrogates in NOM-negative waters, if such improved performance can be experimentally confirmed by future research.

Overall, the findings of this study, although not as broad-based as initially hoped for, adequately support the initial experimental hypothesis that copper and zinc oxide nanoparticles, in doses within allowable concentration limits for copper and zinc ions in drinking water, could be used as an alternative point-of-use household drinking water treatment to improve the microbial quality of the water. However, more research and study must be performed prior to recommendations for widespread implementation and use.

XIII. References

Abad, F. X., Pinto, R. M., Diez, J. M., & Bosch, A. (1994). Disinfection of human enteric viruses in water by copper and silver in combination with low levels of chlorine. *Applied and Environmental Microbiology, 60*(7), 2377-2383.

Armstrong, A. "Characterisation of ionic copper for disinfection of stored water". Master's Technical Report. The University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Environmental Sciences and Engineering (May, 2010)

Aruoja, V., Dubourguier, H. C., Kasemets, K., & Kahru, A. (2009). Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. *Science of the Total Environment, 407*(4), 1461-1468.

Atiyeh, B. S., Costagliola, M., Hayek, S. N., & Dibo, S. A. (2007). Effect of silver on burn wound infection control and healing: review of the literature. *Burns, 33*(2), 139-148.

Badireddy, A.R. Post-Doctoral Fellow. Duke University, Pratt School of Engineering, Department of Civil and Environmental Engineering. Personal communication. (July, 2011)

Black, J. *Microbiology: Principles and Explorations.* Wiley, 8th Edition (January, 2012, 968 pages).

Blanc, D. S., Carrara, P., Zanetti, G., & Francioli, P. (2005). Water disinfection with ozone, copper and silver ions, and temperature increase to control *Legionella* seven years of experience in a university teaching hospital. *Journal of Hospital Infection, 60*(1), 69-72.

Bodnar, W.M. Research Assistant Professor. The University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Environmental Sciences and Engineer. Personal communication (July, 2011)

Box, G.E.P. *Statistics for Experimenters: An Introduction to Design, Data Analysis, and Model Building* Wiley, 1st Edition (June, 1978, 653 pages)

Brayner, R., Ferrari-Iliou, R., Brivois, N., Djediat, S., Benedetti, M. F., & Fiévet, F. (2006). Toxicological impact studies based on Escherichia coli bacteria in ultrafine ZnO nanoparticles colloidal medium. *Nano Letters, 6*(4), 866-870.

Brunet, L., Lyon, D. Y., Hotze, E. M., Alvarez, P. J., & Wiesner, M. R. (2009). Comparative photo-activity and antibacterial properties of C60 fullerenes and titanium dioxide nanoparticles. *Environmental Science & Technology, 43*(12), 4355-4360.

Cachafeiro, S. P., Naveira, I. M., & García, I. G. (2007). Is copper–silver ionisation safe and effective in controlling legionella? *Journal of Hospital Infection, 67*(3), 209-216.

Cervantes, C., & Gutierrez-Corona, F. (1994). Copper resistance mechanisms in bacteria and fungi. *FEMS Microbiology Reviews, 14*(2), 121-137.

Clasen, T., Cairncross, S., Haller, L., Bartram, J., & Walker, D. (2007). Cost-effectiveness of water quality interventions for preventing diarrhoeal disease in developing countries. *Journal of Water and Health, 5*(4), 599-608.

Clescerl, L.S. et al. *Standard Methods for Examination of Water and Wastewater* American Public Health Association, 20th Edition (January 1999, 1325 pages)

Coronell, O. Associate Professor, The University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Environmental Sciences and Engineering. Personal communication (April, 2012)

Domek, M. J., LeChevallier, M. W., Cameron, S. C., & McFeters, G. A. (1984). Evidence for the role of copper in the injury process of coliform bacteria in drinking water. *Applied and Environmental Microbiology, 48*(2), 289-293.

Dongre, A. Professor Emeritus, Mahatma Gandhi Institute of Medical Sciences, Department of Community Medicine (Aanji Field Clinic Site) Sewagram, Maharashtra, India. Personal communication (June, 2010)

Dwidjosiswojo, Z., Richard, J., Moritz, M. M., Dopp, E., Flemming, H. C., & Wingender, J. (2011). Influence of copper ions on the viability and cytotoxicity of *Pseudomonas aeruginosa* under conditions relevant to drinking water environments. *International journal of Hygiene and Environmental Health, 214*(6), 485-492.

Fewtrell, L., Kaufmann, R. B., Kay, D., Enanoria, W., Haller, L., & Colford Jr, J. M. (2005). Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. *The Lancet Infectious Diseases, 5*(1), 42-52.

Fitzgerald, G. P., & Faust, S. L. (1963). Factors affecting the algicidal and algistatic properties of copper.

Applied Microbiology, 11(4), 345-351.

Gadd, G. M., & Griffiths, A. J. (1977). Microorganisms and heavy metal toxicity. *Microbial Ecology, 4*(4), 303-317.

Graebel, W. *Advanced Fluid Mechanics.* Academic Press, 1st Edition (July, 2007, 368 pages)

Heinlaan, M., Ivask, A., Blinova, I., Dubourguier, H. C., & Kahru, A. (2008). Toxicity of nano-sized and bulk ZnO, CuO and TiO_2 to bacteria *Vibrio fischeri* and crustaceans *Daphnia magna* and *Thamnocephalus platyurus. Chemosphere, 71*(7), 1308-1316.

Hermans, M. H. (2006). Silver-Containing Dressings and the Need for Evidence. *AJN The American Journal of Nursing, 106*(12), 60-68.

Hiser, A. et al. "Experiments using copper and zinc". Unpublished data, laboratory of Sobsey, M.D. The University of North Carolina at Chapel Hill, Gillings School of Global Public Health (December, 2008)

Hornstra, L. M., de Leeuw, P. L. A., Moezelaar, R., Wolbert, E. J., De Vries, Y. P., De Vos, W. M., & Abee, T. (2007). Germination of *Bacillus cereus* spores adhered to stainless steel. *International Journal of Food Microbiology, 116*(3), 367-371.

Hunter, P. R. (2009). Household water treatment in developing countries: comparing different intervention types using meta-regression. *Environmental Science & Technology, 43*(23), 8991-8997.

Husmark, U., & Rönner, U. (1993). Adhesion of Bacillus cereus spores to different solid surfaces: cleaned or conditioned with various food agents. *Biofouling, 7*(1), 57-65.

Ilyas, H. and Qazi, I. A. *Titanium Dioxide Nanoparticles in Water Treatment: Phenol Degradation Using Advanced Oxidation Process Employing Titania Nanoparticles.* VDM Verlag, 1st Edition (November, 2010, 72 pages).

Kasemets, K., Ivask, A., Dubourguier, H. C., & Kahru, A. (2009). Toxicity of nanoparticles of ZnO, CuO and TiO_2 to yeast *Saccharomyces cerevisiae. Toxicology in vitro, 23*(6), 1116-1122.

Keithley, J.F. *The Story of Electrical and Magnetic Measurements: From 500 BC to the 19th Century* Wiley, 1st Edition (January, 1999, 468 pages)

Kim, J., Cho, M., Oh, B., Choi, S., & Yoon, J. (2004). Control of bacterial growth in water using synthesized

inorganic disinfectant. *Chemosphere, 55*(5), 775-780.

Komandur, A. "Copper and zinc salts as *E. coli* antagonists in water". Honours Thesis. The University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Environmental Sciences and Engineering (May, 2011)

Kuwahara, J., Suzuki, T., Funakoshi, K., & Sugiura, Y. (1986). Photosensitive DNA cleavage and phage inactivation by copper (II)-camptothecin. *Biochemistry, 25*(6), 1216-1221.

Lehtola, M. J., Miettinen, I. T., Keinänen, M. M., Kekki, T. K., Laine, O., Hirvonen, & Martikainen, P. J. (2004). Microbiology, chemistry and biofilm development in a pilot drinking water distribution system with copper and plastic pipes. *Water Research, 38*(17), 3769-3779.

Li, Q., Xie, R., Li, Y. W., Mintz, E. A., & Shang, J. K. (2007). Enhanced visible-light-induced photo-catalytic disinfection of E. coli by carbon-sensitized nitrogen-doped titanium oxide. *Environmental Science & Technology, 41*(14), 5050-5056.

Liga, M. V., Bryant, E. L., Colvin, V. L., & Li, Q. (2011). Virus inactivation by silver doped titanium dioxide nanoparticles for drinking water treatment. *Water Research, 45*(2), 535-544.

Lin, Y. S. E., Vidic, R. D., Stout, J. E., & Yu, V. L. (1996). Individual and combined effects of copper and silver ions on inactivation of *Legionella pneumophilia*. *Water Research, 30*(8), 1905-1913.

Liu, Y., He, L., Mustapha, A., Li, H., Hu, Z. Q., & Lin, M. (2009). Antibacterial activities of zinc oxide nanoparticles against Escherichia coli O157: H7. *Journal of Applied Microbiology, 107*(4), 1193-1201.

Luna-delRisco, M., Orupõld, K., & Dubourguier, H. C. (2011). Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. *Journal of Hazardous Materials, 189*(1), 603-608.

Maier, R. et al. *Environmental Microbiology*. Academic Press, 2nd Edition (October, 2008, 624 pages)

Malone, A. "Characterization of Ionic Copper and Zinc as Disinfectants in Drinking Water". Master's Technical Report. The University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Environmental Sciences and Engineering (May, 2012)

Manzl, C., Krumschnabel, G., Schwarzbaum, P. J., & Dallinger, R. (2004). Acute toxicity of cadmium and copper in hepatopancreas cells from the Roman snail (*Helix pomatia*). *Comparative Biochemistry and*

Physiology Part C: Toxicology & Pharmacology, 138(1), 45-52.

Mintz, E. D., Reiff, F. M., & Tauxe, R. V. (1995). Safe water treatment and storage in the home: a practical new strategy to prevent waterborne disease. *JAMA-Journal of the American Medical Association-US Edition, 273*(12), 948-953.

Mòdol, J., Sabriã, M., Reynaga, E., Pedro-Botet, M. L., Sopena, N., Tudela, P., Casas & Rey-Joly, C. (2007). Hospital-acquired Legionnaires disease in a university hospital: Impact of the copper-silver ionization system. *Clinical Infectious Diseases, 44*(2), 263-265.

Moore, G. T., & Kellerman, K. F. (1904). *A method of destroying or preventing the growth of algae and certain pathogenic bacteria in water supplies.* U.S. Department of Agriculture Publication (No. 64).

Noyce, J. O., Michels, H., & Keevil, C. W. (2006). Use of copper cast alloys to control Escherichia coli O157 cross-contamination during food processing. *Applied and Environmental Microbiology, 72*(6), 4239-4244.

Pyle, B. H., Broadaway, S. C., & McFeters, G. A. (1992). Efficacy of copper and silver ions with iodine in the inactivation of Pseudomonas cepacia. *Journal of Applied Microbiology, 72*(1), 71-79.

Qin, Y. (2005). Silver-containing alginate fibres and dressings. *International Wound Journal, 2*(2), 172-176.

Raman, N., Sakthivel, A., & Jeyamurugan, R. (2010). Binuclear copper and zinc complexes possessing bio-potential ligands: synthesis, characterization, antimicrobial, SOD mimetic, DNA binding, and cleavage studies. *Journal of Coordination Chemistry, 63*(6), 1080-1096.

Rice, E. W., Adcock, N. J., Sivaganesan, M., & Rose, L. J. (2005). Inactivation of Spores of Bacillus anthracis Sterne, Bacillus cereus, and Bacillus thuringiensis subsp. israelensis by Chlorination. *Applied and Environmental Microbiology, 71*(9), 5587-5589.

Rönner, U., Husmark, U., & Henriksson, A. (1990). Adhesion of Bacillus spores in relation to hydrophobicity. *Journal of Applied Microbiology, 69*(4), 550-556.

Salton, M. R. J., & Marshall, B. (1959). The composition of the spore wall and the wall of vegetative cells of Bacillus subtilis. *Journal of General Microbiology, 21*(2), 415-420.

Samuni, A., Aronovitch, J., Godinger, D., Chevion, M., & Czapski, G. (1983). On the cytotoxicity of vitamin C and metal ions. *European Journal of Biochemistry, 137*(1-2), 119-124.

Shatkin, J.A. *Nanotechnology: Health and Environmental Risks* CRC Press, 1st Edition (April, 2008, 192 pages)

Shih, H. Y., & Lin, Y. E. (2010). Efficacy of copper-silver ionization in controlling biofilm-and plankton-associated waterborne pathogens. *Applied and environmental microbiology, 76*(6), 2032-2035.

Singer, P.C. Daniel A. Okun Distinguished Professor Emeritus, The University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Environmental Sciences and Engineering. Personal communication (February, 2010)

Smieja, J. A. (2011). Household Water Treatments in Developing Countries. *Journal of Chemical Education, 88*(5), 549-553.

Sobsey, M.D. Kenan Distinguished Professor of Environmental Sciences and Engineering. The University of North Carolina at Chapel Hill, Gillings School of Global Public Health, Department of Environmental Sciences and Engineering. Personal communication (February, 2010)

Sobsey, M.D. *Managing Water In the Home: Accelerated Health Gains from improved Water Supply* World Health Organisation, Geneva, 1st Edition (May, 2002, 81 pages)

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

Stout, J. E., & Yu, V. L. (2003). Experiences of the first 16 hospitals using copper-silver ionization for Legionella control: implications for the evaluation of other disinfection modalities. *Infection Control and Hospital Epidemiology, 24*(8), 563-568.

Straub, T. M., Gerba, C. P., Zhou, X., Price, R., & Yahya, M. T. (1995). Synergistic inactivation of *Escherichia coli* and MS-2 coliphage by chloramine and cupric chloride. *Water Research, 29*(3), 811-818.

Sudha, V. P., Singh, K. O., Prasad, S. R., & Venkatasubramanian, P. (2009). Killing of enteric bacteria in drinking water by a copper device for use in the home: laboratory evidence. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 103*(8), 819-822.

Tauveron, G., Slomianny, C., Henry, C., & Faille, C. (2006). Variability among *Bacillus cereus* strains in spore surface properties and influence on their ability to contaminate food surface equipment. *International*

Journal of Food Microbiology, 110(3), 254-262.

Thurman, R. B., Gerba, C. P., & Bitton, G. (1989). The molecular mechanisms of copper and silver ion disinfection of bacteria and viruses. *Critical Reviews in Environmental Science and Technology, 18*(4), 295-315.

U.N. Children's Fund. *Progress on Drinking Water and Sanitation: Update* United Nations, Geneva. (March, 2012)

U.S. Environmental Protection Agency. *Clean Water Act* Office of Water, Washington, D.C. (January, 2012)

U.S. Environmental Protection Agency. *Code of Federal Regulations* Section 40, Part 1-49. Office of water, Washington, D.C. (July, 2002)

U.S. Environmental Protection Agency. *Drinking Water Strategy* Office of Water, Washington, D.C. (January, 2010)

U.S. Environmental Protection Agency. *Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Water Systems Using Surface Water Sources* Office of Water, Washington, D.C. (January, 1989)

Van der Kooij, D., Veenendaal, H. R., & Scheffer, W. J. (2005). Biofilm formation and multiplication of *Legionella* in a model warm water system with pipes of copper, stainless steel and cross-linked polyethylene. *Water Research, 39*(13), 2789-2798.

Vasudevachari, M. B., & Antony, A. (1982). Inhibition of avian myeloblastosis virus reverse transcriptase and virus inactivation by metal complexes of isonicotinic acid hydrazide. *Antiviral Research, 2*(5), 291-300.

Water Supply and Sanitation Collaborative Council. *Household-Centred Environmental Sanitation.* Water Supply and Sanitation Collaborative Council, Geneva (May, 2012)

Wiencek, K. M., Klapes, N. A., & Foegeding, P. M. (1990). Hydrophobicity of Bacillus and Clostridium spores. *Applied and Environmental Microbiology, 56*(9), 2600-2605.

World Health Organisation. *Copper in Drinking Water.* World Health Organisation, Geneva (February, 2004)

World Health Organisation. *Global Annual Assessment of Sanitation and Drinking-Water.* World Health

Organisation, Geneva (July, 2010)

World Health Organisation. *Global Water Supply and Sanitation Assessment.* World Health Organisation, Geneva (September, 2000)

World Health Organisation. *Production of Zinc Tablets and Zinc Oral Solutions.* World Health Organisation, Geneva (March, 2007)

World Health Organisation. *World Health Report* World Health Organisation, Geneva (June, 2012)

World Health Organisation. *Zinc in Drinking Water: Guidelines for Drinking-Water Quality.* World Health Organisation, Geneva, 2nd Edition (April, 1996)

Yahya, M. T., Landeen, L. K., Messina, M. C., Kutz, S. M., Schulze, R., & Gerba, C. P. (1990). Disinfection of bacteria in water systems by using electrolytically generated copper: silver and reduced levels of free chlorine. *Canadian Journal of Microbiology, 36*(2), 109-116.

Zevenhuizen, L. P. T. M., Dolfing, J., Eshuis, E. J., & Scholten-Koerselman, I. J. (1979). Inhibitory effects of copper on bacteria related to the free ion concentration. *Microbial Ecology, 5*(2), 139-146.

XIV. Appendix

Definition of Terms used in ICP-MS Table

1. **Dissolved copper**: the total amount of available copper present in the analysed sample. Available copper is taken to be total copper not removed as aggregates with other material in the water by centrifugation or lost to container walls by adsorption.
2. **Dissolved zinc**: the total amount of available zinc present in the analysed sample. Available zinc is taken to be total zinc not removed by centrifugation as aggregates with other material in the water or lost to container walls by adsorption.
3. **Dose and Organism +** (column 1): Column containing identification values for samples sent for ICP-MS. Column values indicate whether a sample was taken from water dosed with 0.2 or 2 mg/L metal oxide nanoparticles, whether the same contained organisms (Organism +) or did not (Organism -), and from which replicate trial the sample was taken (1, 2, or 3).
4. **Equivalents** (column 2): Column containing numerical values for added dose equivalents in parts per billion. Values were either 200 parts per billion (0.2 mg/L) or 2000 parts per billion (2 mg/L)
5. **Parts Per Billion** (columns 3 and 7): Column containing measured concentrations of dissolved copper or dissolved zinc. Units are parts per billion; to convert to mg/L, divide by 1000.
6. **% Root Std. Dev.** (columns 4 and 8): Column containing per cent root standard deviation values for the measurements in "Parts Per Billion" (columns 3 and 7). Values are equal to $\frac{\sigma_{Array}}{\mu_{Array}} * 100$
7. **Margin of Error** (columns 5 and 9): Column containing the value of one-half the breadth of the 95% confidence interval for the value given in "Parts Per Billion" (columns 3 and 7). For example, a 95% confidence interval lying between 2 and 4 would correspond to a measured value of 3 ± 1. In such a case, the "Margin of Error" would be 1.
8. **% Unaccounted for** (columns 6 and 10): Column containing the per cent of the value given in "Equivalents" (column 2) that was not measured by ICP-MS.
9. **Average Initial Dose** (table 2): Table containing the trial-averaged values for measurements taken at 0 minutes of disinfectant contact time
10. **Average Final Dose** (table 3): Table containing the trial-averaged values for measurements taken at 360 minutes of disinfectant contact time
11. **Average Difference** (table 4): Table containing the trial-averaged values for the difference in measurements taken between 0 and 360 minutes of disinfectant contact time.

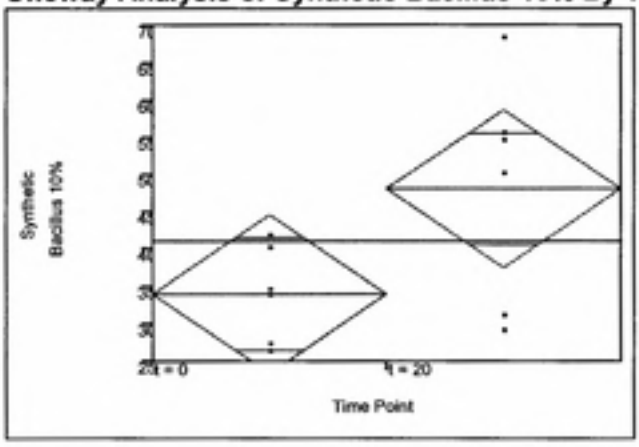
NOM-Negative Water		Dissolved Copper				Dissolved Zinc			
0 Minutes									
Dose And Organism +	**Equivalents**	**Parts per Billion**	**% Root Std. Dev.**	**Margin of Error**	**% Unaccounted For**	**Parts Per Billion**	**% Root Std. Dev.**	**Margin of Error**	**% Unaccounted For**
0.2 mg/L Organism + 1	200	95	0.6	0.57	52.22	141	1.2	1.692	28.65
0.2 mg/L Organism + 2	200	105	4.6	4.83	45.09	165	4.6	7.59	13.71
0.2 mg/L Organism + 3	200	123	32.2	39.606	18.7	199	32.1	63.879	32.44
2 mg/L Organism + 1	2000	814	3.8	30.932	57.75	1321	1.4	18.494	33.03
2 mg/L Organism + 2	2000	1012	10.9	110.308	43.88	1372	1.5	20.58	32.43
2 mg/L Organism + 3	2000	793	3.1	24.583	59.12	1393	2.2	30.646	28.82
0.2 mg/L Organism - 1	200	133	0.7	0.931	33.03	105	5.6	5.88	44.56
0.2 mg/L Organism - 2	200	126	0.8	1.008	36.5	112	3.4	3.808	42.1
0.2 mg/L Organism - 3	200	124	0.4	0.496	37.75	130	8.8	11.44	29.28
2 mg/L Organism - 1	2000	1239	0.6	7.434	37.68	1108	1	11.08	44.05
2 mg/L Organism - 2	2000	1294	2.9	37.526	33.42	1105	4.5	49.725	42.26
2 mg/L Organism - 3	2000	1252	1.5	18.78	36.46	1072	4.7	50.384	43.88
360 Minutes									
0.2 mg/L Organism + 1	200	96	2.8	2.688	50.66	135	39.1	52.785	6.11
0.2 mg/L Organism + 2	200	101	1.3	1.313	48.84	142	39.7	56.374	0.81
0.2 mg/L Organism + 3	200	98	0.4	0.392	50.8	134	6.9	9.246	28.38
2 mg/L Organism + 1	2000	827	2.1	17.367	57.78	1404	29.4	412.776	9.16
2 mg/L Organism + 2	2000	876	2.2	19.272	55.24	2158	1.3	28.054	-9.3
2 mg/L Organism + 3	2000	804	1.2	9.648	59.32	2183	0.2	4.366	-9.37
0.2 mg/L Organism - 1	200	193	0.4	0.772	3.11	91	5.9	5.369	51.82
0.2 mg/L Organism - 2	200	180	0.2	0.36	9.82	74	6.8	5.032	65.52
0.2 mg/L Organism - 3	200	182	0.3	0.546	8.73	128	10.5	13.44	29.28
2 mg/L Organism - 1	2000	1263	0.9	11.367	36.28	1132	1.5	16.98	42.55
2 mg/L Organism - 2	2000	1548	14.7	227.556	11.22	1257	7.1	89.247	32.69
2 mg/L Organism - 3	2000	1326	1.3	17.238	32.84	1076	2.7	29.052	44.75
Average Initial Dose									
0.2 mg/L Organism +	200	107.6666667	12.46666667	13.42244444	38.67	168.3333333	12.63333333	21.26611111	24.93333333
2 mg/L Organism +	2000	873	5.933333333	51.798	53.58333333	1362	1.7	23.154	31.42666667
0.2 mg/L Organism -	200	127.6666667	0.633333333	0.808555556	35.76	115.6666667	5.933333333	6.862888889	38.64666667
2 mg/L Organism -	2000	1261.666667	1.666666667	21.02777778	35.85333333	1095	3.4	37.23	43.39666667
Average Final Dose									
0.2 mg/L Organism +	200	98.33333333	1.5	1.475	50.1	137	28.56666667	39.13633333	11.76666667
2 mg/L Organism +	2000	835.6666667	1.833333333	15.32055556	57.44666667	1915	10.3	197.245	-3.17
0.2 mg/L Organism -	200	185	0.3	0.555	7.22	97.66666667	7.733333333	7.552888889	48.87333333
2 mg/L Organism -	2000	1379	5.633333333	77.68366667	26.78	1155	3.766666667	43.505	39.9666667

Average Difference								
Dose And Organism	**Cu (ppb)**	**% Diff. (0-360 mins)**	**Margin of Error**	**0 In CI?**	**Zn (ppb)**	**% Diff. (0-360 mins)**	**Margin of Error**	**0 in CI?**
0.2 mg/L Organism +	-9.333333333	-4.666666667	16.46633333	Yes	-31.33333333	-15.66666667	63.85533333	Yes
2 mg/L Organism +	-37.33333333	-1.866666667	70.70333333	Yes	553	27.65	171.6386667	No
0.2 mg/L Organism -	57.33333333	28.66666667	1.371	No	-18	-9	14.98966667	No
2 mg/L Organism -	117.3333333	5.866666667	106.6336667	No	60	3	82.156	Yes

NOM-Amended Water		Dissolved Copper				Dissolved Zinc			
0 Minutes									
Dose And Organism +	**Equivalents**	**Parts Per Billion**	**% Root Std. Dev.**	**Margin of Error**	**% Unaccounted For**	**Parts Per Billion**	**% Root Std. Dev.**	**Margin of Error**	**% Unaccounted For**
0.2 mg/L Organism + 1	200	159	0.3	0.477	20.26	137	1	1.37	30.82
0.2 mg/L Organism + 2	200	104	9.3	9.672	43.16	384	3.7	14.208	-99.1
0.2 mg/L Organism + 3	200	101	22.6	22.826	38.09	338	1.8	6.084	-72.04
2 mg/L Organism + 1	2000	365	11.8	43.07	79.6	1144	1	11.44	42.23
2 mg/L Organism + 2	2000	423	11	46.53	76.52	1132	2	22.64	44.53
2 mg/L Organism + 3	2000	446	22.4	99.904	72.7	1241	1.3	16.133	37.14
0.2 mg/L Organism - 1	200	89	36.4	32.396	39.3	270	5	13.5	-41.75
0.2 mg/L Organism - 2	200	139	25.4	35.306	12.85	395	5.3	20.935	-107.97
0.2 mg/L Organism - 3	200	136	72.3	98.328	-17.16	326	2.9	9.454	-67.73
2 mg/L Organism - 1	2000	441	18.4	81.144	73.89	1287	0.7	9.009	35.2
2 mg/L Organism - 2	2000	420	14	58.8	76.06	1140	4	45.6	40.72
2 mg/L Organism - 3	2000	475	2.2	10.45	75.73	1140	4.3	49.02	40.55
360 Minutes									
0.2 mg/L Organism + 1	200	135	39.1	52.785	6.11	318	4.7	14.946	-66.47
0.2 mg/L Organism + 2	200	142	39.7	56.374	0.81	290	4.5	13.05	-51.53
0.2 mg/L Organism + 3	200	134	6.9	9.246	28.38	378	11.4	43.092	-110.55
2 mg/L Organism + 1	2000	441	18.4	81.144	73.89	1132	1.5	16.98	42.55
2 mg/L Organism + 2	2000	420	14	58.8	76.06	1257	7.1	89.247	32.69
2 mg/L Organism + 3	2000	475	2.2	10.45	75.73	1076	2.7	29.052	44.75
0.2 mg/L Organism - 1	200	51	40	20.4	64.3	207	42.8	88.596	-47.8
0.2 mg/L Organism - 2	200	41	13.9	5.699	76.65	304	9.7	29.488	-37.26
0.2 mg/L Organism - 3	200	51	41.7	21.267	63.87	394	8.4	33.096	-113.55
2 mg/L Organism - 1	2000	505	21.5	108.575	69.32	1363	5.4	73.602	28.17
2 mg/L Organism - 2	2000	391	37.3	145.843	73.16	1784	9.2	164.128	2.59
2 mg/L Organism - 3	2000	453	24	108.72	71.91	1393	5.9	82.187	26.24
Average Initial Dose									
0.2 mg/L + Organisms	200	121.3333333	10.73333333	13.02311111	33.83666667	286.3333333	2.166666667	6.203888889	-46.77333333
2 mg/L Organism +	2000	411.3333333	15.06666667	61.97422222	76.27333333	1172.333333	1.433333333	16.80344444	41.3
0.2 mg/L Organism -	200	121.3333333	44.7	54.236	11.66333333	330.3333333	4.4	14.53466667	-72.48333333
2 mg/L Organism -	2000	445.3333333	11.53333333	51.36177778	75.22666667	1189	3	35.67	38.82333333
Average Final Dose									
0.2 mg/L Organism +	200	137	28.56666667	39.13633333	11.76666667	328.6666667	6.866666667	22.56844444	-76.18333333
2 mg/L Organism +	2000	445.3333333	11.53333333	51.36177778	75.22666667	1155	3.766666667	43.505	39.99666667
0.2 mg/L Organism -	200	47.66666667	31.86666667	15.18977778	68.27333333	301.6666667	20.3	61.23833333	-66.20333333
2 mg/L Organism -	2000	449.6666667	27.6	124.108	71.46333333	1513.333333	6.833333333	103.4111111	19

Average Difference								
Dose And Organism +	**Cu (ppb)**	**% Diff. (0-360 mins)**	**Margin of Error**	**0 Within CI?**	**Zn (ppb)**	**% Diff. (0-360 mins)**	**Margin of Error**	**0 in CI?**
0.2 mg/L Organism +	15.66666667	7.833333333	50.46	Yes	42.33333333	21.16666667	30.91666667	No
2 mg/L Organism +	34	1.7	113.2993333	Yes	-17.33333333	-0.866666667	61.83066667	Yes
0.2 mg/L Organism -	-73.66666667	-36.83333333	71.132	No	-28.66666667	-14.33333333	65.023	Yes
2 mg/L Organism -	4.333333333	0.216666667	171.1773333	Yes	324.3333333	16.21666667	141.182	No

Fit Y by X Group
Oneway Analysis of Synthetic Bacillus 10% By Time Point



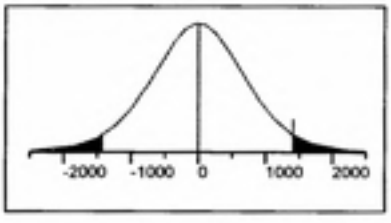
Oneway Anova
Summary of Fit

Rsquare	0.30451
Adj Rsquare	0.234961
Root Mean Square Error	1166.391
Mean of Response	4098.485
Observations (or Sum Wgts)	12

t Test
t = 20-t = 0

Assuming equal variances

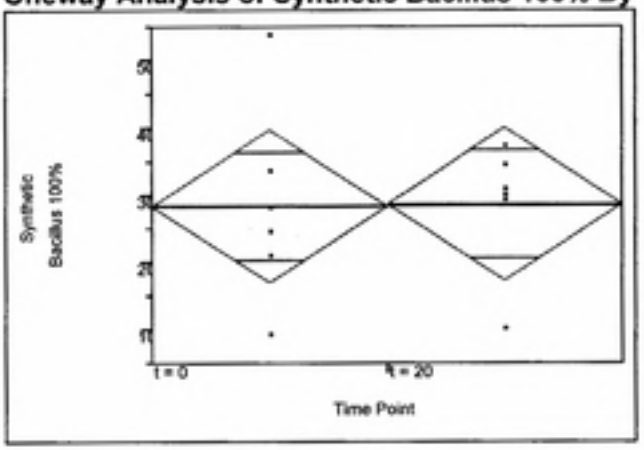
Difference	1409.1	t Ratio	2.092452
Std Err Dif	673.4	DF	10
Upper CL Dif	2909.6	Prob > |t|	0.0629
Lower CL Dif	-91.4	Prob > t	0.0314*
Confidence	0.95	Prob < t	0.9686



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	1	5956612	5956612	4.3784	0.0629
Error	10	13604683	1360468		
C. Total	11	19561295			

Oneway Analysis of Synthetic Bacillus 100% By Time Point



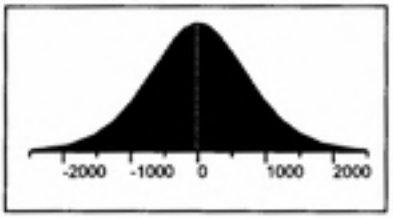
Oneway Anova
Summary of Fit

Rsquare	0.000175
Adj Rsquare	-0.09981
Root Mean Square Error	1253.865
Mean of Response	2848.485
Observations (or Sum Wgts)	12

t Test
t = 20-t = 0

Assuming equal variances

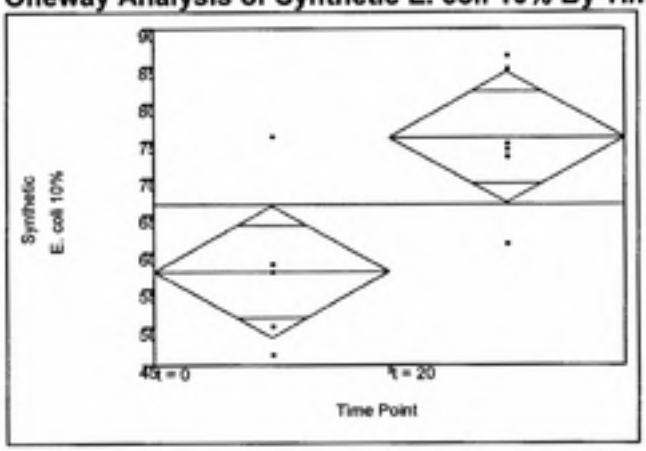
Difference	30.3	t Ratio	0.04186
Std Err Dif	723.9	DF	10
Upper CL Dif	1643.3	Prob > |t|	0.9674
Lower CL Dif	-1582.7	Prob > t	0.4837
Confidence	0.95	Prob < t	0.5163



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	1	2755	2755	0.0018	0.9674
Error	10	15721763	1572176		
C. Total	11	15724518			

Oneway Analysis of Synthetic E. coli 10% By Time Point



Oneway Anova
Summary of Fit

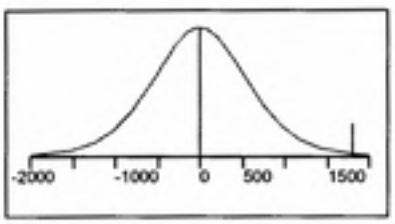
Rsquare	0.51243
Adj Rsquare	0.463673
Root Mean Square Error	963.3075
Mean of Response	6643.939
Observations (or Sum Wgts)	12

t Test

t = 20-t = 0

Assuming equal variances

Difference	1803.03	t Ratio	3.241893
Std Err Dif	556.17	DF	10
Upper CL Dif	3042.25	Prob > |t|	0.0088*
Lower CL Dif	563.82	Prob > t	0.0044*
Confidence	0.95	Prob < t	0.9956



Analysis of Variance

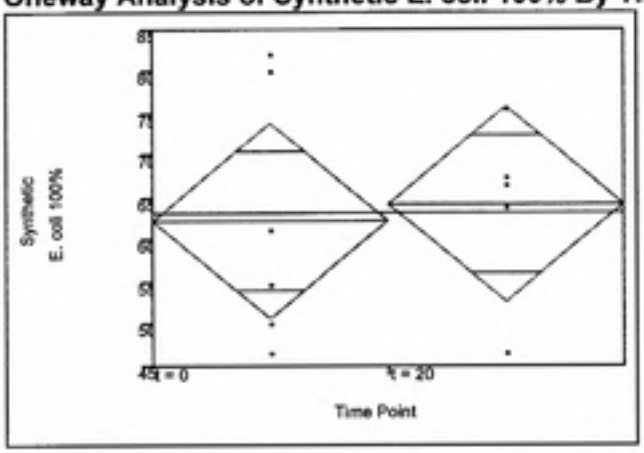
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	1	9752755	9752755	10.5099	0.0088*
Error	10	9279614	927961		
C. Total	11	19032369			

Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
t = 0	6	5742.42	393.27	4866.2	6618.7
t = 20	6	7545.45	393.27	6669.2	8421.7

Std Error uses a pooled estimate of error variance

Oneway Analysis of Synthetic E. coli 100% By Time Point



Oneway Anova
Summary of Fit

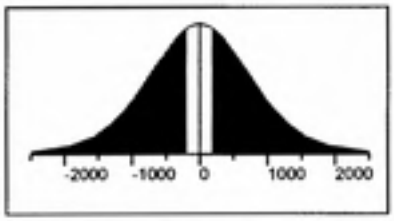
Rsquare	0.00711
Adj Rsquare	-0.09218
Root Mean Square Error	1274.944
Mean of Response	6325.758
Observations (or Sum Wgts)	12

t Test

t = 20-t = 0

Assuming equal variances

Difference	197.0	t Ratio	0.267589
Std Err Dif	736.1	DF	10
Upper CL Dif	1837.1	Prob > |t|	0.7945
Lower CL Dif	-1443.1	Prob > t	0.3972
Confidence	0.95	Prob < t	0.6028



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	1	116391	116391	0.0716	0.7945
Error	10	16254821	1625482		
C. Total	11	16371212			

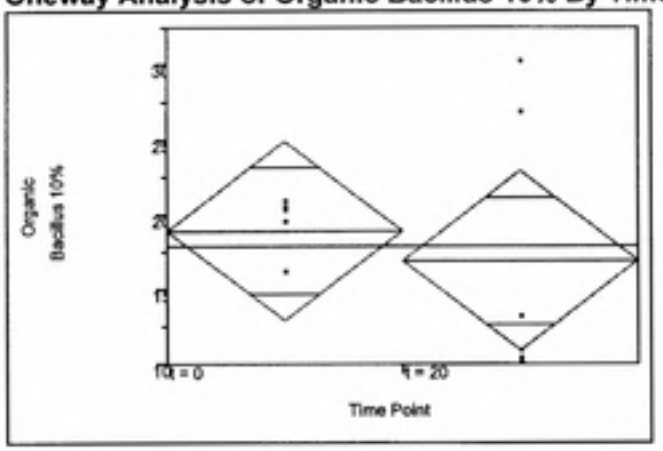
Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
t = 0	6	6227.27	520.49	5067.5	7387.0

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
t = 20	6	6424.24	520.49	5264.5	7584.0

Std Error uses a pooled estimate of error variance

Oneway Analysis of Organic Bacillus 10% By Time Point



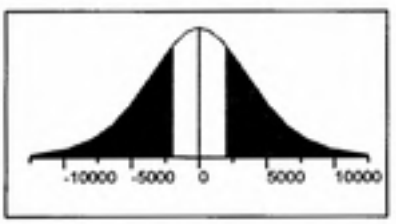
Oneway Anova
Summary of Fit

Rsquare	0.026933
Adj Rsquare	-0.07037
Root Mean Square Error	6584.459
Mean of Response	17893.94
Observations (or Sum Wgts)	12

t Test
t = 20-t = 0

Assuming equal variances

Difference	-2000	t Ratio	-0.5261
Std Err Dif	3802	DF	10
Upper CL Dif	6470	Prob > |t|	0.6103
Lower CL Dif	-10470	Prob > t	0.6949
Confidence	0.95	Prob < t	0.3051



Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Time Point	1	12000000	12000000	0.2768	0.6103
Error	10	433550964	43355096		
C. Total	11	445550964			

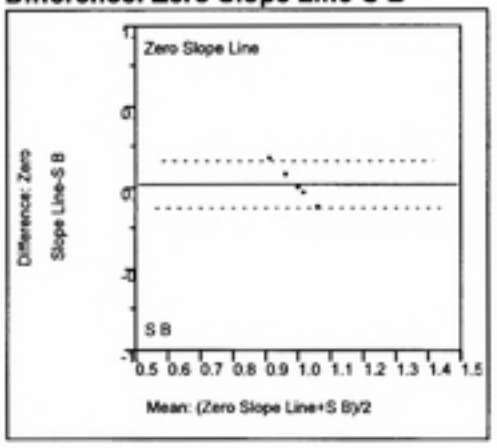
Means for Oneway Anova

Level	Number	Mean	Std Error	Lower 95%	Upper 95%
t = 0	6	18893.9	2688.1	12904	24883
t = 20	6	16893.9	2688.1	10904	22883

Std Error uses a pooled estimate of error variance

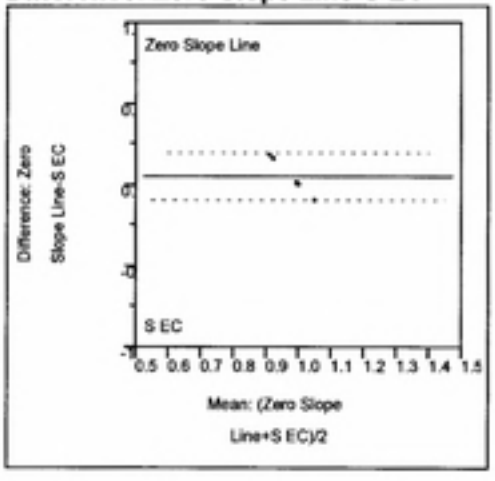
Matched Pairs

Difference: Zero Slope Line-S B



Zero Slope Line	1	t-Ratio	0.379628
S B	0.98039	DF	4
Mean Difference	0.01961	Prob > |t|	0.7235
Std Error	0.05166	Prob > t	0.3618
Upper 95%	0.16306	Prob < t	0.6382
Lower 95%	-0.1238		
N	5		
Correlation	0		

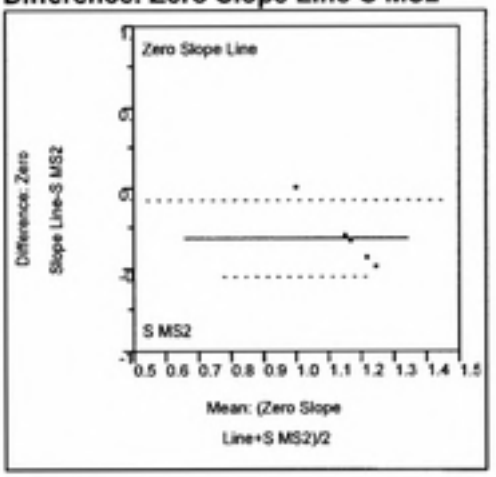
Difference: Zero Slope Line-S EC



Zero Slope Line	1	t-Ratio	0.935816
S EC	0.95095	DF	4
Mean Difference	0.04905	Prob > |t|	0.4023
Std Error	0.05241	Prob > t	0.2012

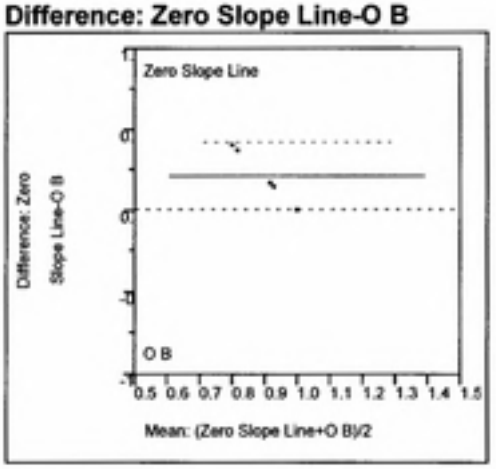
Upper 95%	0.19456	Prob < t	0.7988
Lower 95%	-0.0965		
N	5		
Correlation	0		

Difference: Zero Slope Line-S MS2



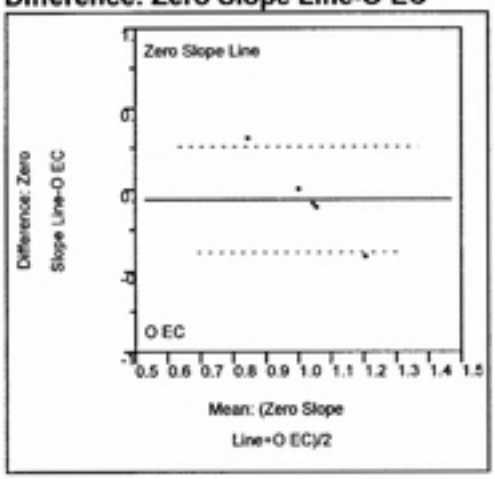
Zero Slope Line	1	t-Ratio	-3.6955
S MS2	1.31226	DF	4
Mean Difference	-0.3123	Prob > |t|	0.0209*
Std Error	0.0845	Prob > t	0.9895
Upper 95%	-0.0777	Prob < t	0.0105*
Lower 95%	-0.5469		
N	5		
Correlation	0		

Difference: Zero Slope Line-O B



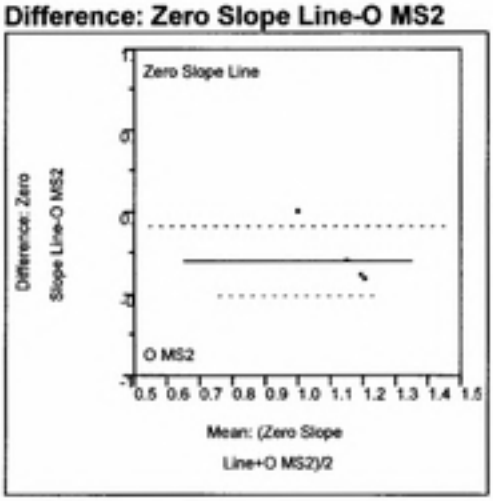
Zero Slope Line	1	t-Ratio	2.861298
O B	0.78454	DF	4
Mean Difference	0.21546	Prob > |t|	0.0459*
Std Error	0.0753	Prob > t	0.0229*
Upper 95%	0.42453	Prob < t	0.9771
Lower 95%	0.00639		
N	5		
Correlation	0		

Difference: Zero Slope Line-O EC



Zero Slope Line	1	t-Ratio	-0.50762
O EC	1.05904	DF	4
Mean Difference	-0.059	Prob > |t|	0.6384
Std Error	0.1163	Prob > t	0.6808
Upper 95%	0.26386	Prob < t	0.3192
Lower 95%	-0.3819		
N	5		
Correlation	0		

Difference: Zero Slope Line-O MS2



Zero Slope Line	1	t-Ratio	-3.88643
O MS2	1.29809	DF	4
Mean Difference	-0.2981	Prob > |t|	0.0177*
Std Error	0.0767	Prob > t	0.9911
Upper 95%	-0.0851	Prob < t	0.0089*
Lower 95%	-0.5111		
N	5		
Correlation	0		

Nonlinear Fit

Response: Log(Survival Ratio): Organic Bacillus 10%, Predictor: Synthetic Bacillus 10% (Use for Dose Or Water)

Control Panel

Converged in Gradient

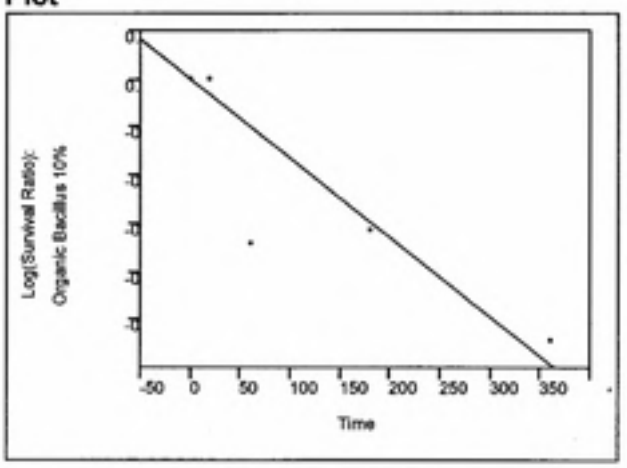
Criterion	Current	Stop Limit
Iteration	1	60
Obj Change	0.0044252414	1e-15
Relative Gradient	6.975834e-17	0.000001
Gradient	1.271057e-14	0.000001

Parameter	Current Value	Lock
k1	-0.001993	X
b0	0.0003452804	

SSE
0.0630651894N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
k1	-0.001993	-0.003	-0.001
b0	0.0003452804	5e-101	2e-100

Solution

SSE	DFE	MSE	RMSE
0.0630651894	4	0.0157663	0.1255639

Parameter	Estimate	ApproxStdErr
k1	-0.001993	0
b0	0.0003452804	0.00030818

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic Bacillus 100%, Predictor: Synthetic Bacillus 100% (Water Only)

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	1	60
Obj Change	0.3010810095	1e-15
Relative Gradient	2.267146e-16	0.000001
Gradient	4.130937e-14	0.000001

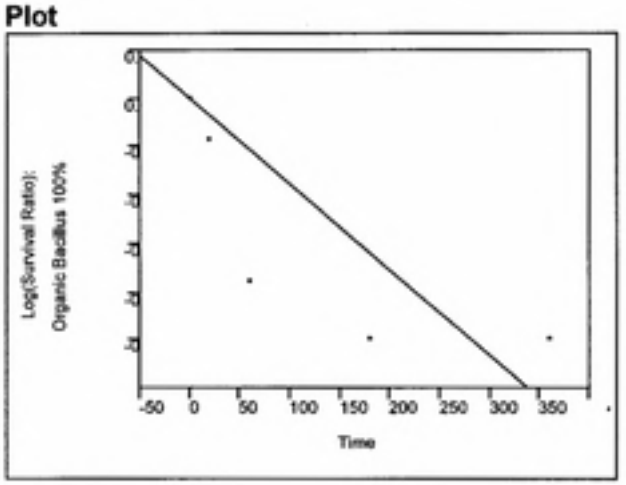
Parameter	Current Value	Lock
b0	0.0028480338	

SSE
0.1279600439N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
b0	0.0028480338	5e-101	2e-100

Solution

SSE	DFE	MSE	RMSE
0.1279600439	4	0.03199	0.1788575

Parameter	Estimate	ApproxStdErr
b0	0.0028480338	0.00043899

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic E. coli 10%, Predictor: Synthetic E. coli 10% (Use for Dose OR Water)

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	1	60
Obj Change	0.090944415	1e-15
Relative Gradient	6.975834e-17	0.000001
Gradient	1.271057e-14	0.000001

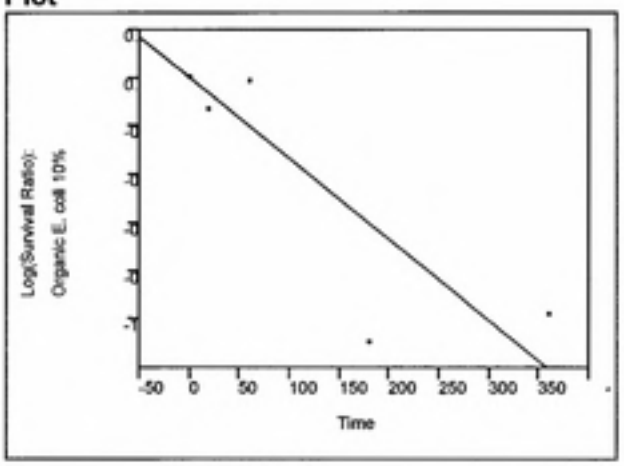
Parameter	Current Value	Lock
k1	-0.001784	X
b0	-0.001565278	

SSE
0.3349866235N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
k1	-0.001784	-0.0027	-0.0009
b0	-0.001565278	5e-101	2e-100

Solution

SSE	DFE	MSE	RMSE
0.3349866235	4	0.0837467	0.2893901

Parameter	Estimate	ApproxStdErr
k1	-0.001784	0
b0	-0.001565278	0.00071028

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic E. coli 100%, Predictor: Synthetic E. coli 100% (Water Only)

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	1	60
Obj Change	0.1306357892	1e-15
Relative Gradient	1.046375e-16	0.000001
Gradient	1.906586e-14	0.000001

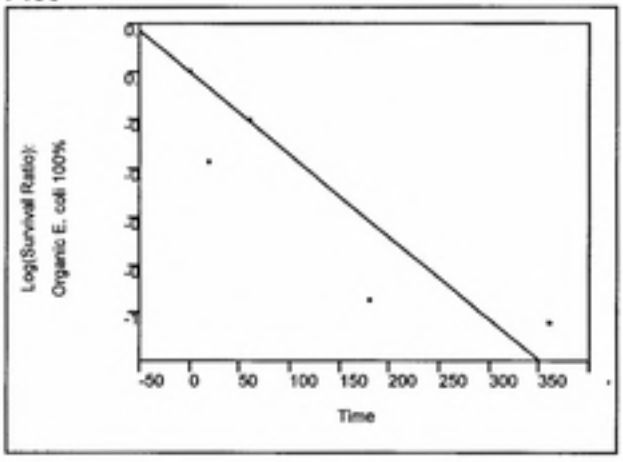
Parameter	Current Value	Lock
b0	0.0018760074	

SSE
0.2400172348N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
b0	0.0018760074	5e-101	2e-100

Solution

SSE	DFE	MSE	RMSE
0.2400172348	4	0.0600043	0.2449578

Parameter	Estimate	ApproxStdErr
b0	0.0018760074	0.00060123

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Synthetic Bacillus 100%, Predictor: Synthetic Bacillus Dose Comparison

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	1	60
Obj Change	0.2560983475	1e-15
Relative Gradient	8.719793e-18	0.000001
Gradient	1.588822e-15	0.000001

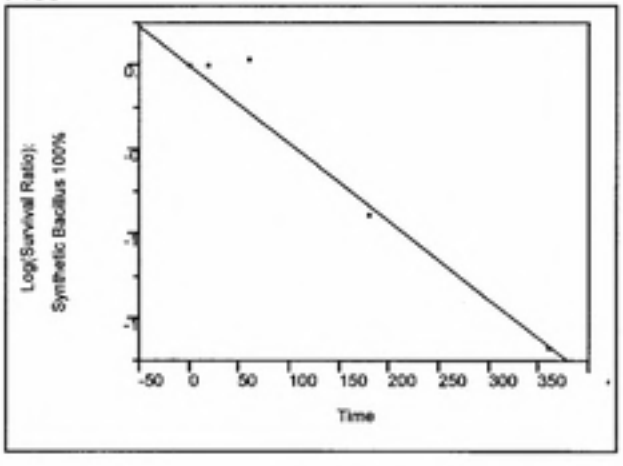
Parameter	Current Value	Lock
k1	-0.001993	X
b0	-0.002626678	

SSE
0.1079352125N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
k1	-0.001993	-0.003	-0.001
b0	-0.002626678	5e-101	2e-100

Solution

SSE	DFE	MSE	RMSE
0.1079352125	4	0.0269838	0.1642675

Parameter	Estimate	ApproxStdErr
k1	-0.001993	0
b0	-0.002626678	0.00040318

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Synthetic E. coli 100%, Predictor: Synthetic E. coli Dose Comparison

Control Panel

Converged in Gradient

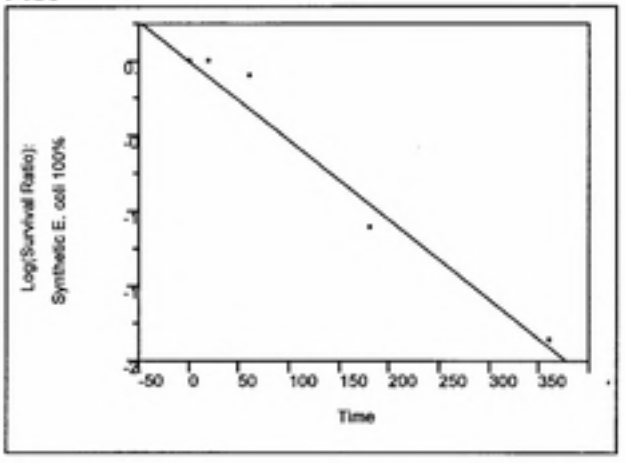
Criterion	Current	Stop Limit
Iteration	1	60
Obj Change	0.4578240328	1e-15
Relative Gradient	2.96473e-16	0.000001
Gradient	5.401994e-14	0.000001

Parameter	Current Value	Lock
k1	-0.001784	X
b0	-0.003511986	

SSE
0.0880559439N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
k1	-0.001784	-0.0027	-0.0009
b0	-0.003511986	5e-101	2e-100

Solution

SSE	DFE	MSE	RMSE
0.0880559439	4	0.022014	0.1483711

Parameter	Estimate	ApproxStdErr
k1	-0.001784	0
b0	-0.003511986	0.00036416

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic Bacillus 100%, Predictor: Organic Bacillus Dose

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	1	60
Obj Change	0.0005704266	1e-15
Relative Gradient	3.487917e-17	0.000001
Gradient	6.355287e-15	0.000001

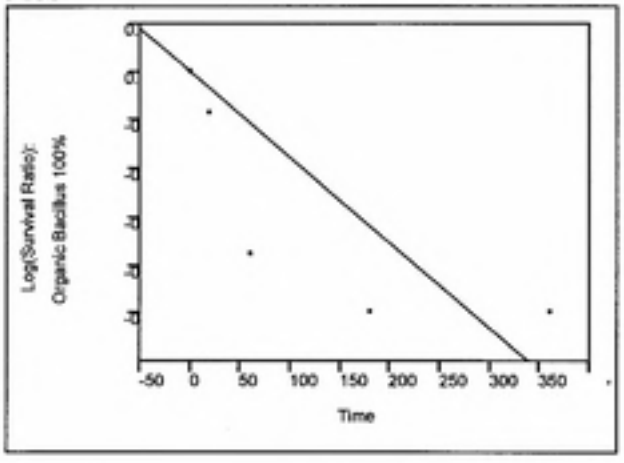
Parameter	Current Value	Lock
k1	-0.001648	X
b0	-0.000123966	

SSE
0.1279600439N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
k1	-0.001648	-0.0025	-0.0008
b0	-0.000123966	5e-101	2e-100

Solution

SSE	DFE	MSE	RMSE
0.1279600439	4	0.03199	0.1788575

Parameter	Estimate	ApproxStdErr
k1	-0.001648	0
b0	-0.000123966	0.00043899

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic E. coli 100%, Predictor: Organic E. coli Dose

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	1	60
Obj Change	0.0001870766	1e-15
Relative Gradient	1.046375e-16	0.000001
Gradient	1.906586e-14	0.000001

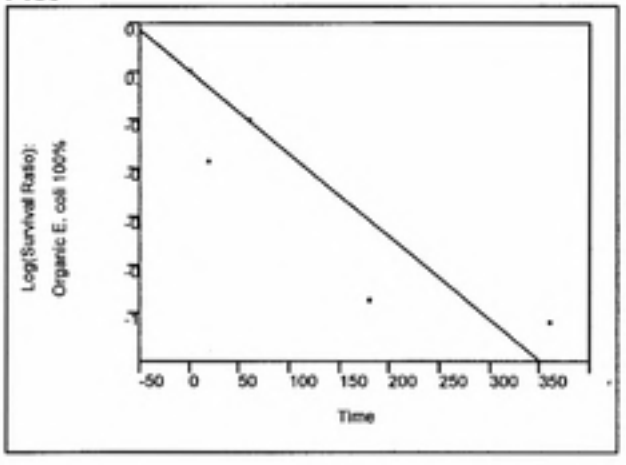
Parameter	Current Value	Lock
k1	-0.003349	X
b0	-0.000070993	

SSE
0.2400172348N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
k1	-0.003349	-0.005	-0.0017
b0	-0.000070993	5e-101	2e-100

Solution

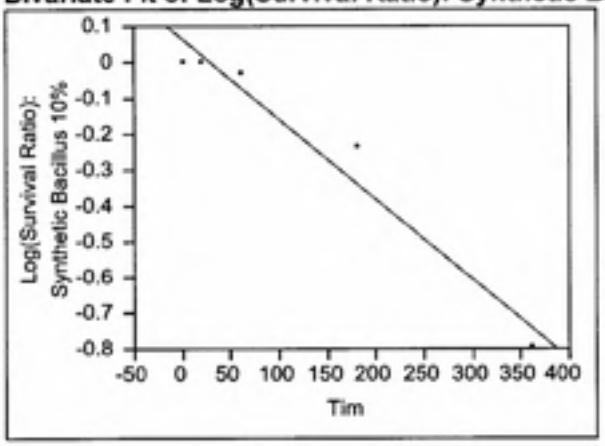
SSE	DFE	MSE	RMSE
0.2400172348	4	0.0600043	0.2449578

Parameter	Estimate	ApproxStdErr
k1	-0.003349	0
b0	-0.000070993	0.00060123

Solved By:
Analytic Gauss-Newton

Fit Y by X Group

Bivariate Fit of Log(Survival Ratio): Synthetic Bacillus 10% By Time



Linear

Linear Fit

Log(Survival Ratio): Synthetic Bacillus 10% = 0.0643989 - 0.0022335*Time

Summary of Fit

RSquare	0.958443
RSquare Adj	0.94459
Root Mean Square Error	0.080158
Mean of Response	-0.21255
Observations (or Sum Wgts)	5

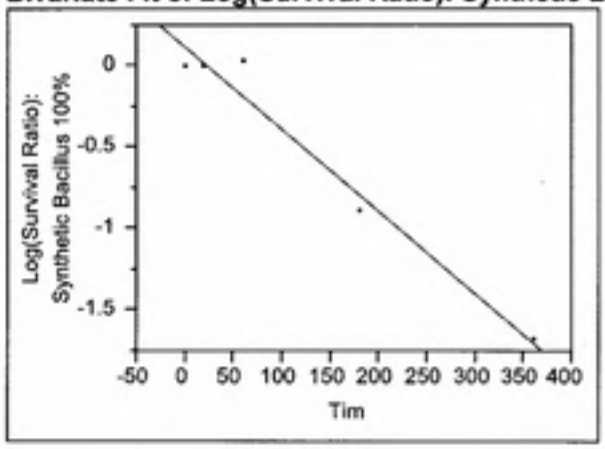
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.44457030	0.444570	69.1898
Error	3	0.01927613	0.006425	Prob > F
C. Total	4	0.46384643		0.0036*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0643989	0.048925	1.32	0.2796
Time	-0.002233	0.000269	-8.32	0.0036*

Bivariate Fit of Log(Survival Ratio): Synthetic Bacillus 100% By Time



Linear

Linear Fit

Log(Survival Ratio): Synthetic Bacillus 100% = 0.1161589 - 0.0050535*Time

Summary of Fit

RSquare	0.969452
RSquare Adj	0.95927
Root Mean Square Error	0.154613
Mean of Response	-0.51048
Observations (or Sum Wgts)	5

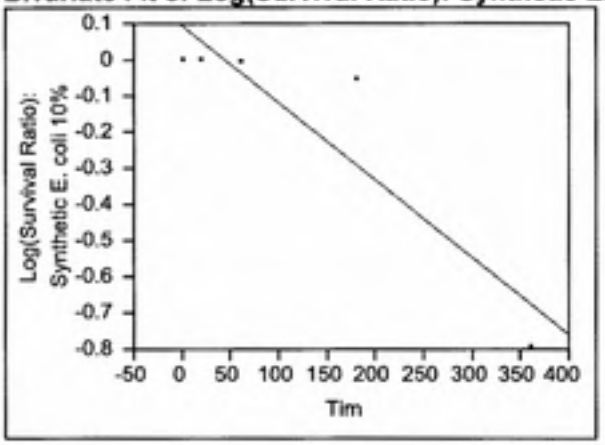
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	2.2759585	2.27596	95.2074
Error	3	0.0717157	0.02391	Prob > F
C. Total	4	2.3476723		0.0023*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.1161589	0.094369	1.23	0.3061
Time	-0.005054	0.000518	-9.76	0.0023*

Bivariate Fit of Log(Survival Ratio): Synthetic E. coli 10% By Time



Linear

Linear Fit

Log(Survival Ratio): Synthetic E. coli 10% = 0.0946231 - 0.0021371*Time

Summary of Fit

RSquare	0.830748
RSquare Adj	0.774331
Root Mean Square Error	0.166257
Mean of Response	-0.17037
Observations (or Sum Wgts)	5

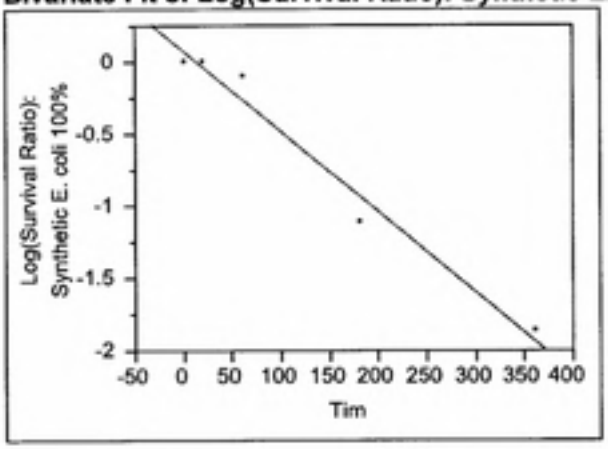
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.40701976	0.407020	14.7250
Error	3	0.08292401	0.027641	Prob > F
C. Total	4	0.48994377		0.0312*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0946231	0.101475	0.93	0.4199
Time	-0.002137	0.000557	-3.84	0.0312*

Bivariate Fit of Log(Survival Ratio): Synthetic E. coli 100% By Time



Linear

Linear Fit

Log(Survival Ratio): Synthetic E. coli 100% = 0.0756006 - 0.0055783*Time

Summary of Fit

RSquare	0.97445
RSquare Adj	0.965933
Root Mean Square Error	0.155685
Mean of Response	-0.61611
Observations (or Sum Wgts)	5

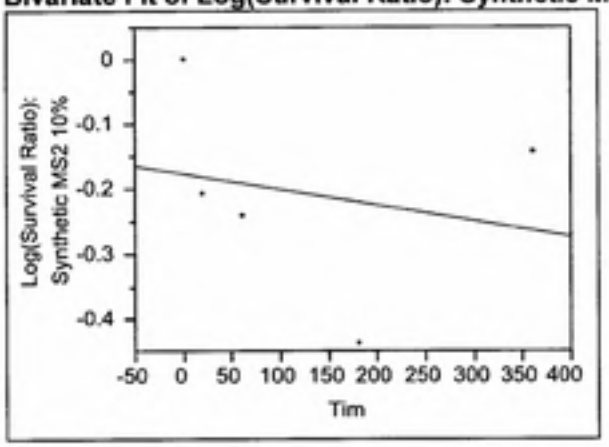
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	2.7732345	2.77323	114.4172
Error	3	0.0727138	0.02424	Prob > F
C. Total	4	2.8459483		0.0017*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.0756006	0.095023	0.80	0.4844
Time	-0.005578	0.000522	-10.70	0.0017*

Bivariate Fit of Log(Survival Ratio): Synthetic MS2 10% By Time



Linear

Linear Fit

Log(Survival Ratio): Synthetic MS2 10% = -0.177105 - 0.0002401*Time

Summary of Fit

RSquare	0.050762
RSquare Adj	-0.26565
Root Mean Square Error	0.178988
Mean of Response	-0.20688
Observations (or Sum Wgts)	5

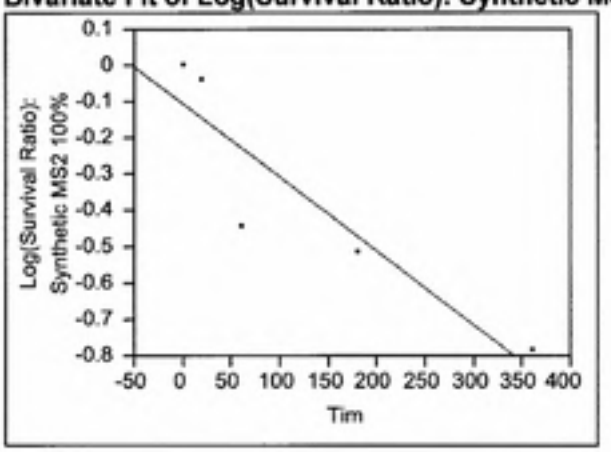
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.00513960	0.005140	0.1604
Error	3	0.09610960	0.032037	Prob > F
C. Total	4	0.10124920		0.7156

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.177105	0.109246	-1.62	0.2034
Time	-0.00024	0.0006	-0.40	0.7156

Bivariate Fit of Log(Survival Ratio): Synthetic MS2 100% By Time



Linear

Linear Fit

Log(Survival Ratio): Synthetic MS2 100% = -0.105731 - 0.0020287*Time

Summary of Fit

RSquare	0.831637
RSquare Adj	0.775516
Root Mean Square Error	0.157325
Mean of Response	-0.35729
Observations (or Sum Wgts)	5

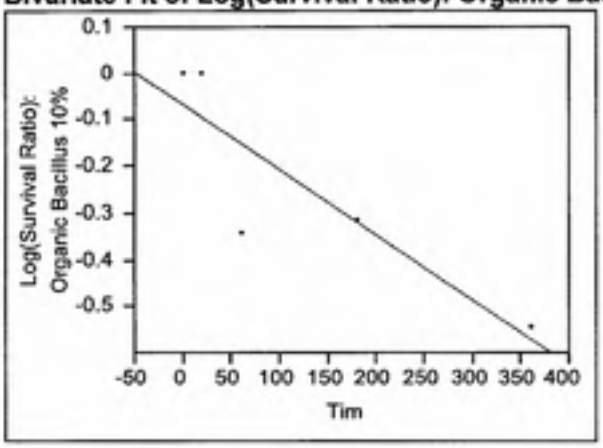
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.36677670	0.366777	14.8187
Error	3	0.07425301	0.024751	Prob > F
C. Total	4	0.44102972		0.0310*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.105731	0.096024	-1.10	0.3513
Time	-0.002029	0.000527	-3.85	0.0310*

Bivariate Fit of Log(Survival Ratio): Organic Bacillus 10% By Time



Linear Fit

Log(Survival Ratio): Organic Bacillus 10% = -0.067627 - 0.0013951*Time

Summary of Fit

RSquare	0.77352
RSquare Adj	0.698027
Root Mean Square Error	0.130114
Mean of Response	-0.24062
Observations (or Sum Wgts)	5

Analysis of Variance

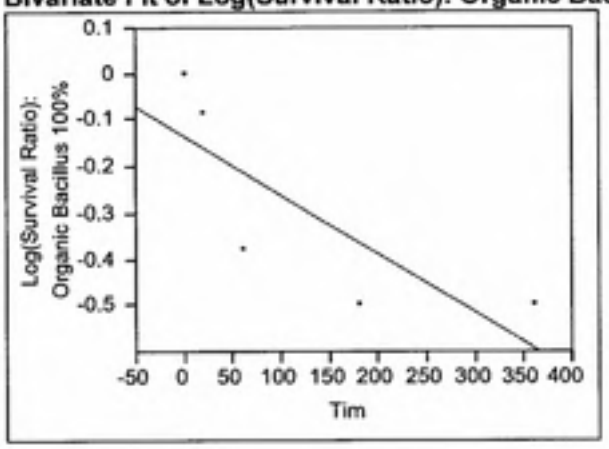
Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.17346414	0.173464	10.2462
Error	3	0.05078875	0.016930	Prob > F
C. Total	4	0.22425289		0.0493*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.067627	0.079415	-0.85	0.4570

Term	Estimate	Std Error	t Ratio	Prob>|t|
Time	-0.001395	0.000436	-3.20	0.0493*

Bivariate Fit of Log(Survival Ratio): Organic Bacillus 100% By Time



Linear

Linear Fit

Log(Survival Ratio): Organic Bacillus 100% = -0.135877 - 0.0012645*Time

Summary of Fit

RSquare	0.645077
RSquare Adj	0.526769
Root Mean Square Error	0.161659
Mean of Response	-0.29267
Observations (or Sum Wgts)	5

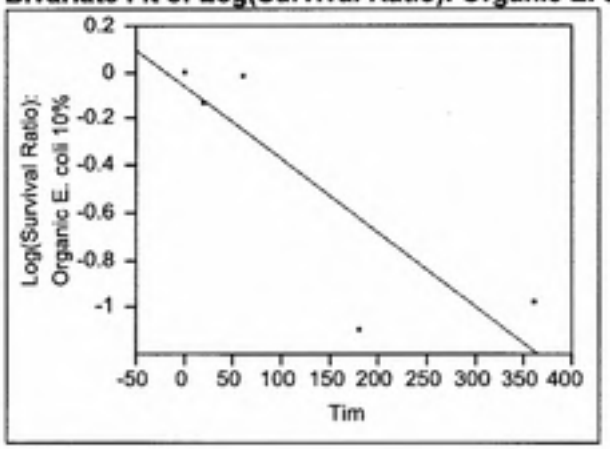
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.14249358	0.142494	5.4525
Error	3	0.07840043	0.026133	Prob > F
C. Total	4	0.22089401		0.1017

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.135877	0.098669	-1.38	0.2622
Time	-0.001264	0.000542	-2.34	0.1017

Bivariate Fit of Log(Survival Ratio): Organic E. coli 10% By Time



Linear

Linear Fit

Log(Survival Ratio): Organic E. coli 10% = -0.059519 - 0.003127*Time

Summary of Fit

RSquare	0.728064
RSquare Adj	0.637419
Root Mean Square Error	0.329382
Mean of Response	-0.44726
Observations (or Sum Wgts)	5

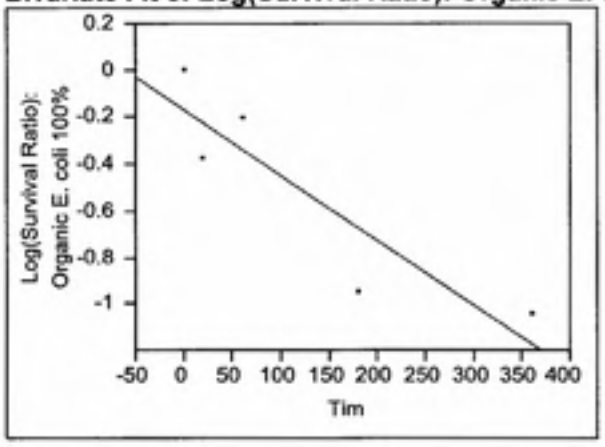
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.8714137	0.871414	8.0320
Error	3	0.3254772	0.108492	Prob > F
C. Total	4	1.1968909		0.0660

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.059519	0.201039	-0.30	0.7865
Time	-0.003127	0.001103	-2.83	0.0660

Bivariate Fit of Log(Survival Ratio): Organic E. coli 100% By Time



Linear

Linear Fit

Log(Survival Ratio): Organic E. coli 100% = -0.169352 - 0.0027875*Time

Summary of Fit

RSquare	0.809431
RSquare Adj	0.745909
Root Mean Square Error	0.233117
Mean of Response	-0.515
Observations (or Sum Wgts)	5

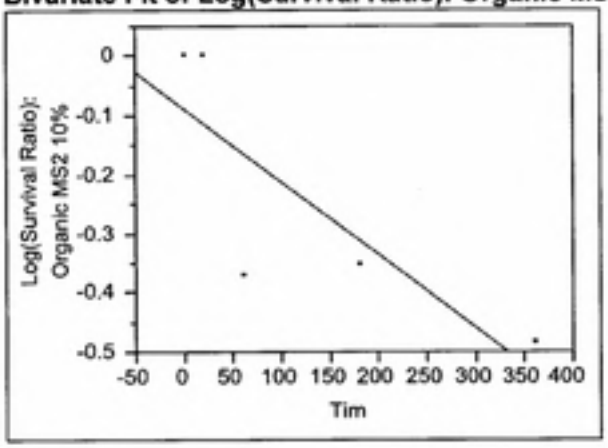
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.69246280	0.692463	12.7424
Error	3	0.16303009	0.054343	Prob > F
C. Total	4	0.85549290		0.0376*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.169352	0.142284	-1.19	0.3196
Time	-0.002787	0.000781	-3.57	0.0376*

Bivariate Fit of Log(Survival Ratio): Organic MS2 10% By Time



—— Linear

Linear Fit

Log(Survival Ratio): Organic MS2 10% = -0.088635 - 0.0012362*Time

Summary of Fit

RSquare	0.664467
RSquare Adj	0.552623
Root Mean Square Error	0.151402
Mean of Response	-0.24192
Observations (or Sum Wgts)	5

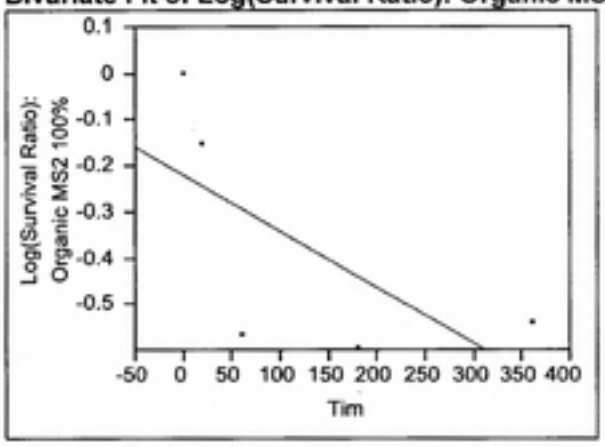
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.13618364	0.136184	5.9410
Error	3	0.06876803	0.022923	Prob > F
C. Total	4	0.20495167		0.0927

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.088635	0.092409	-0.96	0.4082
Time	-0.001236	0.000507	-2.44	0.0927

Bivariate Fit of Log(Survival Ratio): Organic MS2 100% By Time



Linear

Linear Fit

Log(Survival Ratio): Organic MS2 100% = -0.2205 - 0.0012156*Time

Summary of Fit

RSquare	0.436957
RSquare Adj	0.249276
Root Mean Square Error	0.237826
Mean of Response	-0.37123
Observations (or Sum Wgts)	5

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	1	0.13168507	0.131685	2.3282
Error	3	0.16968365	0.056561	Prob > F
C. Total	4	0.30136872		0.2245

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	-0.2205	0.145158	-1.52	0.2261
Time	-0.001216	0.000797	-1.53	0.2245

Nonlinear Fit

Response: Log(Survival Ratio): Synthetic Bacillus 100%, Predictor: General Hom Preduction

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	23	60
Obj Change	8.509351e-13	1e-15
Relative Gradient	6.6165698e-7	0.000001
Gradient	3.8313211e-7	0.000001

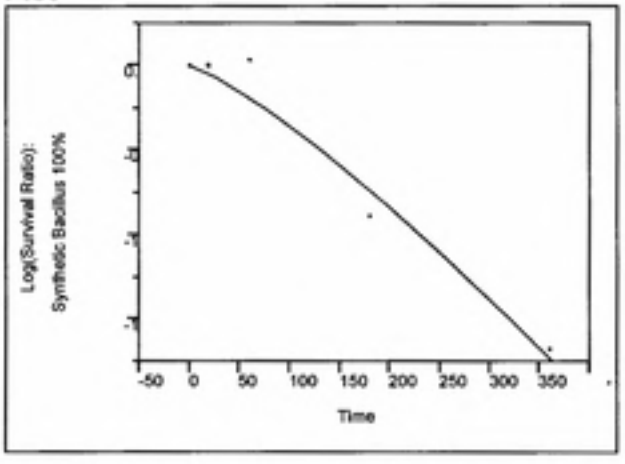
Parameter	Current Value	Lock
Slope	-0.001209205	
Time Exponent	1.2344321866	

SSE
0.0782331309N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
Slope	-0.001209205	-3.8e-5	-1.3e-5
Time Exponent	1.2344321866	1	2.6366

Solution

SSE	DFE	MSE	RMSE
0.0782331309	3	0.0260777	0.1614859

Parameter	Estimate	ApproxStdErr
Slope	-0.001209205	0.00193427
Time Exponent	1.2344321866	0.27725488

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Synthetic Bacillus 10%, Predictor: General Hom Production

Control Panel

Converged in Gradient

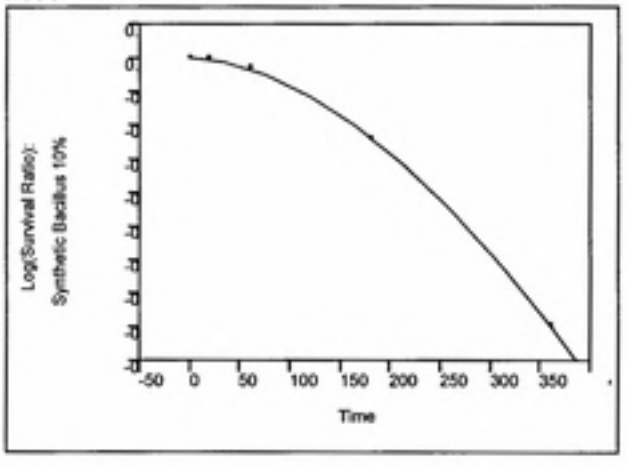
Criterion	Current	Stop Limit
Iteration	34	60
Obj Change	1.2985051e-9	1e-15
Relative Gradient	1.409597e-7	0.000001
Gradient	0.0002923147	0.000001

Parameter	Current Value	Lock
Slope	-2.555365e-5	
Time Exponent	1.7577332186	

SSE
0.0000432268N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Slope	-2.555365e-5	-0.0533	-0.0178
Time Exponent	1.7577332186	0	0.789

Solution

SSE	DFE	MSE	RMSE
0.0000432268	3	0.0000144	0.0037959

Parameter	Estimate	ApproxStdErr
Slope	-2.555365e-5	3.37043e-6
Time Exponent	1.7577332186	0.02262048

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Synthetic E. coli 10%, Predictor: General Hom Preduction

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	244	500
Obj Change	1.6443338e-9	1e-15
Relative Gradient	4.7045342e-9	0.000001
Gradient	17.285871904	0.000001

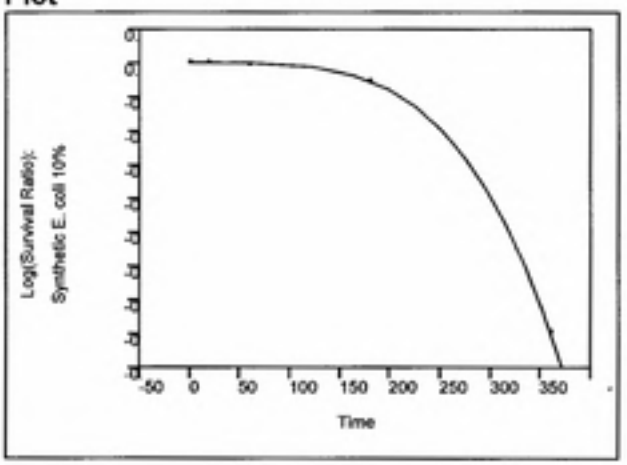
Parameter	Current Value	Lock
Slope	-9.28587e-11	
Time Exponent	3.8855331436	

SSE
5.3150023e-6N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
Slope	-9.28587e-11	-0.0018	-0.0006
Time Exponent	3.8855331436	1	1.85165

Solution

SSE	DFE	MSE	RMSE
5.3150023e-6	3	1.7717e-6	0.001331

Parameter	Estimate	ApproxStdErr
Slope	-9.28587e-11	1.9532e-11
Time Exponent	3.8855331436	0.03575313

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Synthetic E. coli 100%, Predictor: General Horn Preduction

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	20	1000
Obj Change	3.891838e-12	1e-15
Relative Gradient	1.2550309e-6	0.000001
Gradient	5.6420611e-7	0.000001

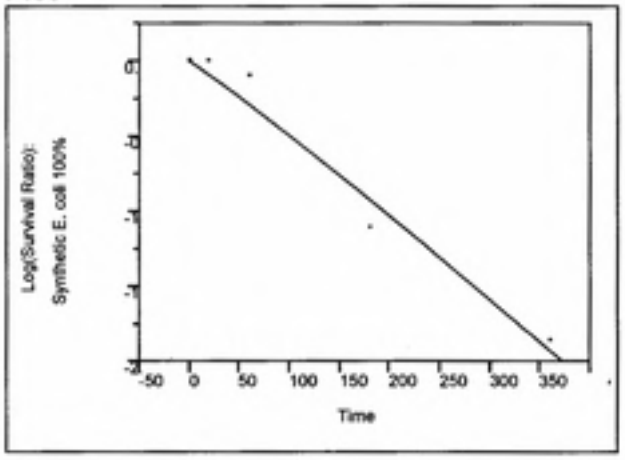
Parameter	Current Value	Lock
Slope	-0.003768382	
Time Exponent	1.0596153637	

SSE
0.0851136482N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
Slope	-0.003768382	-1e-10	0
Time Exponent	1.0596153637	1	5.8283

Solution

SSE	DFE	MSE	RMSE
0.0851136482	3	0.0283712	0.1684376

Parameter	Estimate	ApproxStdErr
Slope	-0.003768382	0.00462118
Time Exponent	1.0596153637	0.21388351

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Synthetic MS2 100%, Predictor: General Hom Preduction

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	5	60
Obj Change	2.179156e-12	1e-15
Relative Gradient	3.9377121e-7	0.000001
Gradient	1.2399632e-7	0.000001

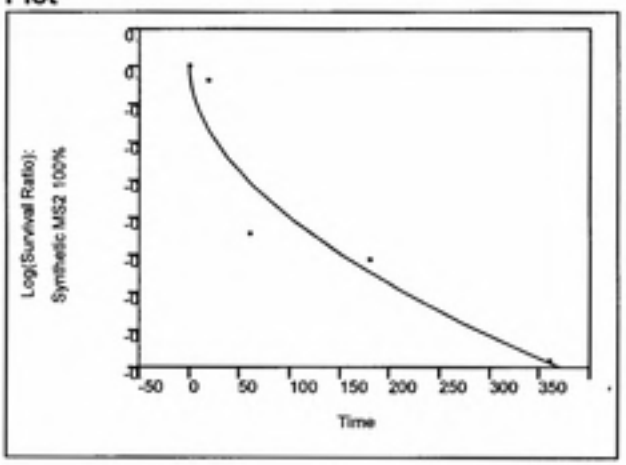
Parameter	Current Value	Lock
Slope	-0.035540479	
Time Exponent	0.5263155205	

SSE
0.0370312855N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
Slope	-0.035540479	-0.0795	-0.0265
Time Exponent	0.5263155205	0.25804	0.77412

Solution

SSE	DFE	MSE	RMSE
0.0370312855	3	0.0123438	0.1111025

Parameter	Estimate	ApproxStdErr
Slope	-0.035540479	0.03099635
Time Exponent	0.5263155205	0.15895802

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic Bacillus 10%, Predictor: General Hom Preduction

Control Panel

Converged in Gradient

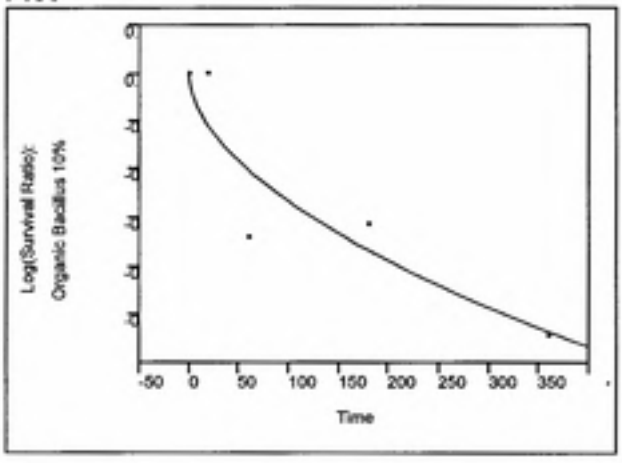
Criterion	Current	Stop Limit
Iteration	23	60
Obj Change	1.358086e-11	1e-15
Relative Gradient	9.761734e-7	0.000001
Gradient	2.029562e-7	0.000001

Parameter	Current Value	Lock
Slope	-0.022466846	
Time Exponent	0.5391569315	

SSE
0.0346778928N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Slope	-0.022466846	-0.0057	0
Time Exponent	0.5391569315	1	1.58942

Solution

SSE	DFE	MSE	RMSE
0.0346778928	3	0.0115593	0.1075142

Parameter	Estimate	ApproxStdErr
Slope	-0.022466846	0.02847882
Time Exponent	0.5391569315	0.2305968

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic Bacillus 100%, Predictor: General Horn Preduction

Control Panel

Converged in Gradient

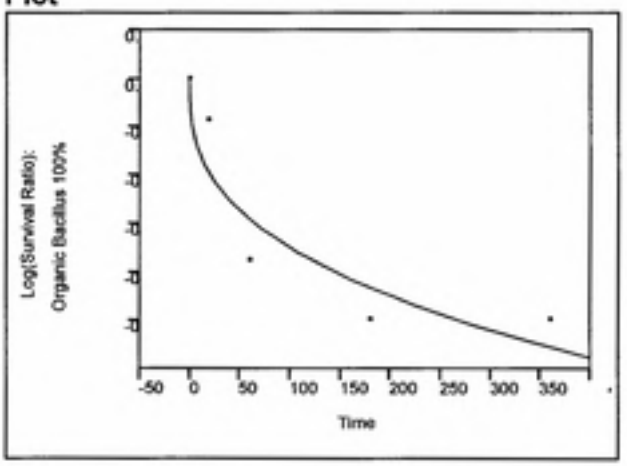
Criterion	Current	Stop Limit
Iteration	10	60
Obj Change	5.52605e-12	1e-15
Relative Gradient	1.169849e-6	0.000001
Gradient	3.4853704e-7	0.000001

Parameter	Current Value	Lock
Slope	-0.068197788	
Time Exponent	0.356333366	

SSE
0.0269599283N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Slope	-0.068197788	-0.0337	0
Time Exponent	0.356333366	0.26958	1

Solution

SSE	DFE	MSE	RMSE
0.0269599283	3	0.0089866	0.0947979

Parameter	Estimate	ApproxStdErr
Slope	-0.068197788	0.05176629
Time Exponent	0.356333366	0.14287226

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic E. coli 10%, Predictor: General Hom Preduction

Control Panel

Converged in Gradient

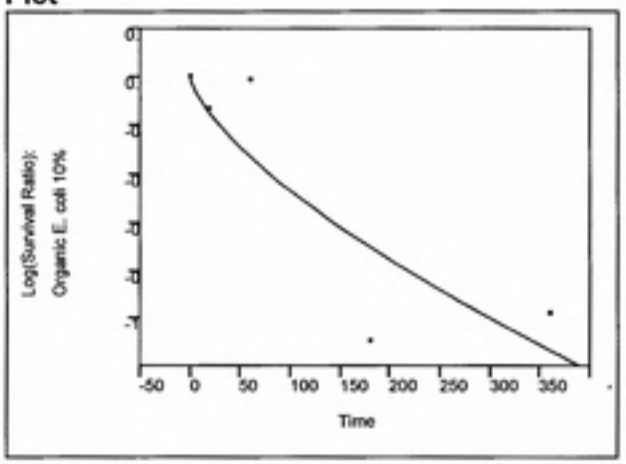
Criterion	Current	Stop Limit
Iteration	16	60
Obj Change	6.971254e-12	1e-15
Relative Gradient	2.6583132e-6	0.000001
Gradient	9.2537889e-7	0.000001

Parameter	Current Value	Lock
Slope	-0.019617677	
Time Exponent	0.689550557	

SSE
0.2777421759N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Slope	-0.019617677	-0.1023	-0.0341
Time Exponent	0.689550557	0.17817	1

Solution

SSE	DFE	MSE	RMSE
0.2777421759	3	0.0925807	0.3042708

Parameter	Estimate	ApproxStdErr
Slope	-0.019617677	0.04328634
Time Exponent	0.689550557	0.39423659

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic E. coli 100%, Predictor: General Hom Preduction

Control Panel

Converged in Gradient

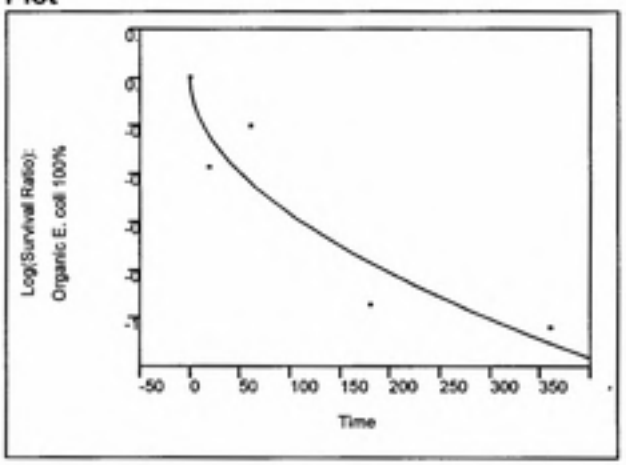
Criterion	Current	Stop Limit
Iteration	3	60
Obj Change	8.728487e-10	1e-15
Relative Gradient	3.6804265e-8	0.000001
Gradient	2.0163476e-8	0.000001

Parameter	Current Value	Lock
Slope	-0.053017167	
Time Exponent	0.5160787597	

SSE
0.1066698757N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Slope	-0.053017167	-0.0294	0
Time Exponent	0.5160787597	0.34478	1

Solution

SSE	DFE	MSE	RMSE
0.1066698757	3	0.0355566	0.1885646

Parameter	Estimate	ApproxStdErr
Slope	-0.053017167	0.05482172
Time Exponent	0.5160787597	0.18875862

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Log(Survival Ratio): Organic MS2 100%, Predictor: General Hom Preduction

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	0	60
Obj Change	.	1e-15
Relative Gradient	1.4487221e-6	0.000001
Gradient	5.9434778e-7	0.000001

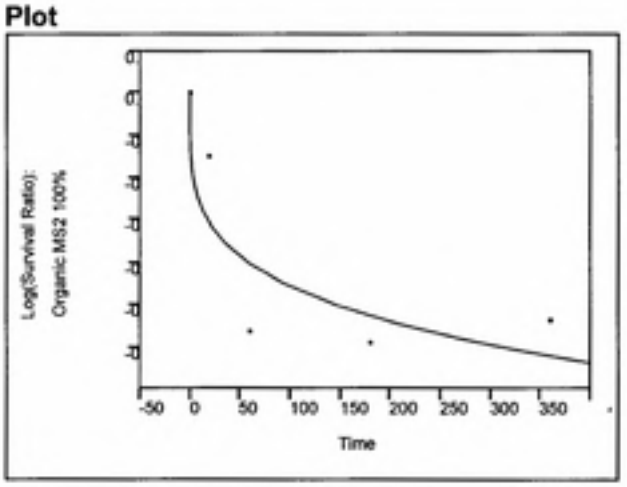
Parameter	Current Value	Lock
Slope	-0.152283717	
Time Exponent	0.2393159914	

SSE
0.0621092969N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
Slope	-0.152283717	-0.0533	-0.0178
Time Exponent	0.2393159914	0.26316	0.78947

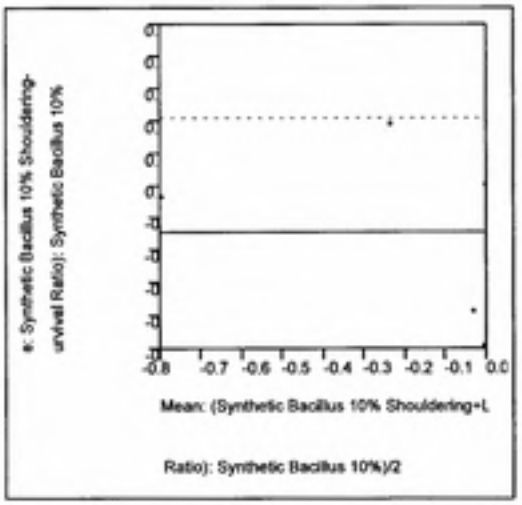
Solution

SSE	DFE	MSE	RMSE
0.0621092969	3	0.0207031	0.1438857

Parameter	Estimate	ApproxStdErr
Slope	-0.152283717	0.12319722
Time Exponent	0.2393159914	0.15720667

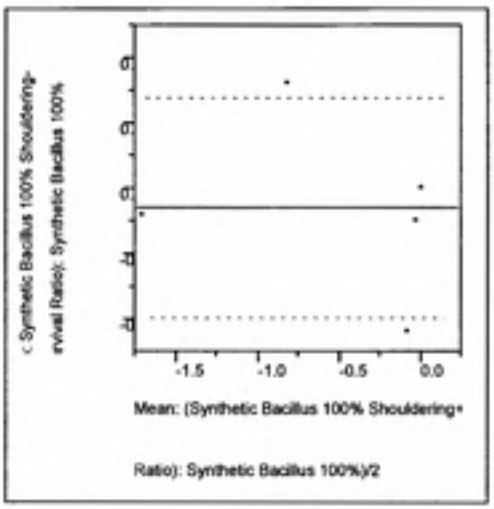
Solved By:
Analytic Gauss-Newton

Matched Pairs
Difference: Synthetic Bacillus 10% Shouldering-Log(Survival Ratio): Synthetic Bacillus 10%



Synthetic Bacillus 10% Shouldering	-0.214	t-Ratio	-1.14425
Log(Survival Ratio): Synthetic Bacillus 10%	-0.2126	DF	4
Mean Difference	-0.0015	Prob > |t|	0.3163
Std Error	0.00128	Prob > t	0.8418
Upper 95%	0.00208	Prob < t	0.1582
Lower 95%	-0.005		
N	5		
Correlation	0.99997		

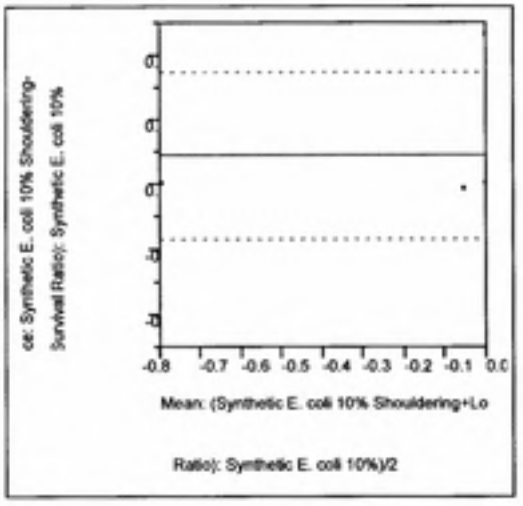
Difference: Synthetic Bacillus 100% Shouldering-Log(Survival Ratio): Synthetic Bacillus 100%



Synthetic Bacillus 100% Shouldering	-0.5407	t-Ratio	-0.49881
Log(Survival Ratio): Synthetic Bacillus 100%	-0.5105	DF	4
Mean Difference	-0.0303	Prob > |t|	0.6441
Std Error	0.06068	Prob > t	0.6780
Upper 95%	0.13822	Prob < t	0.3220

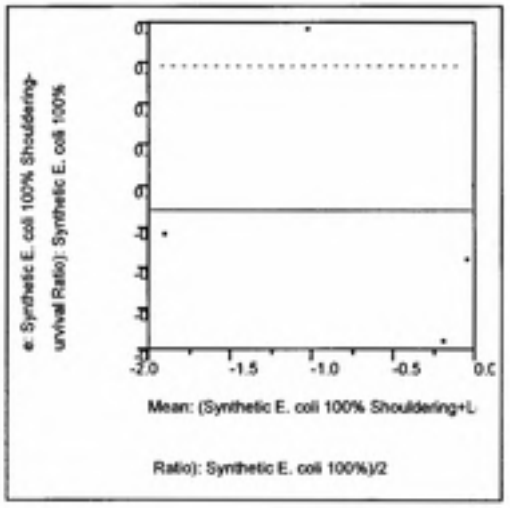
Lower 95%	-0.1988
N	5
Correlation	0.98488

Difference: Synthetic E. coli 10% Shouldering-Log(Survival Ratio): Synthetic E. coli 10%



Synthetic E. coli 10% Shouldering	-0.1699	t-Ratio	0.950845
Log(Survival Ratio): Synthetic E. coli 10%	-0.1704	DF	4
Mean Difference	0.00044	Prob > |t|	0.3955
Std Error	0.00047	Prob > t	0.1978
Upper 95%	0.00174	Prob < t	0.8022
Lower 95%	-0.0008		
N	5		
Correlation	1		

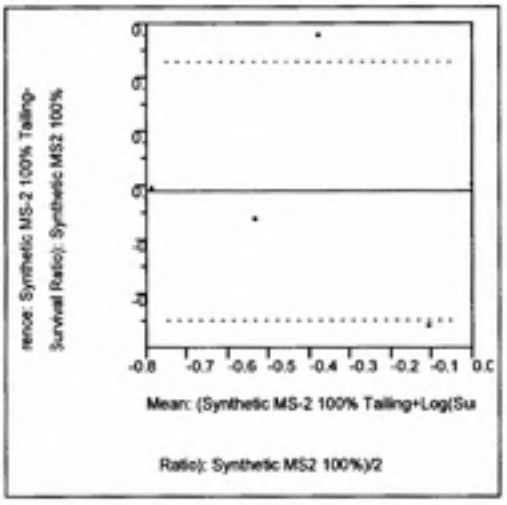
Difference: Synthetic E. coli 100% Shouldering-Log(Survival Ratio): Synthetic E. coli 100%



Synthetic E. coli 100% Shouldering	-0.646	t-Ratio	-0.47069
Log(Survival Ratio): Synthetic E. coli 100%	-0.6161	DF	4
Mean Difference	-0.0299	Prob > |t|	0.6624

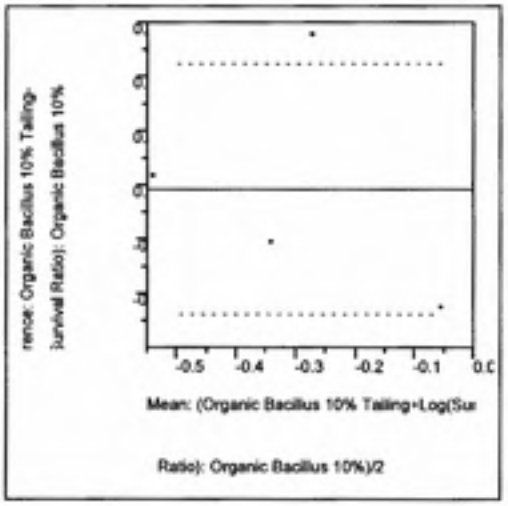
Std Error	0.0635	Prob > t	0.6688
Upper 95%	0.14642	Prob < t	0.3312
Lower 95%	-0.2062		
N	5		
Correlation	0.98638		

Difference: Synthetic MS-2 100% Tailing-Log(Survival Ratio): Synthetic MS2 100%



Synthetic MS-2 100% Tailing	-0.3625	t-Ratio	-0.12161
Log(Survival Ratio): Synthetic MS2 100%	-0.3573	DF	4
Mean Difference	-0.0052	Prob > |t|	0.9091
Std Error	0.04295	Prob > t	0.5455
Upper 95%	0.11403	Prob < t	0.4545
Lower 95%	-0.1245		
N	5		
Correlation	0.95753		

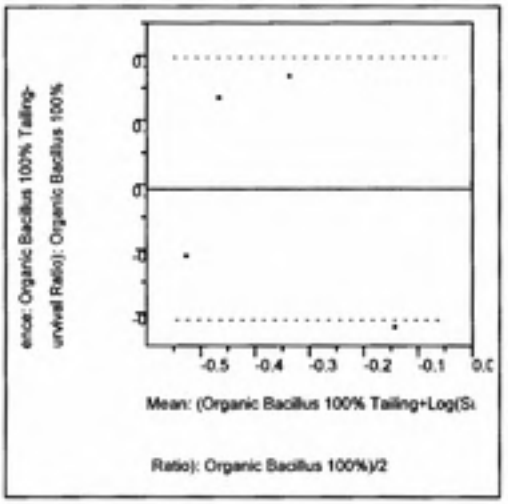
Difference: Organic Bacillus 10% Tailing-Log(Survival Ratio): Organic Bacillus 10%



Organic Bacillus 10% Tailing	-0.2447	t-Ratio	-0.0977
Log(Survival Ratio): Organic Bacillus 10%	-0.2406	DF	4
Mean Difference	-0.0041	Prob > |t|	0.9269
Std Error	0.04159	Prob > t	0.5366
Upper 95%	0.11141	Prob < t	0.4634

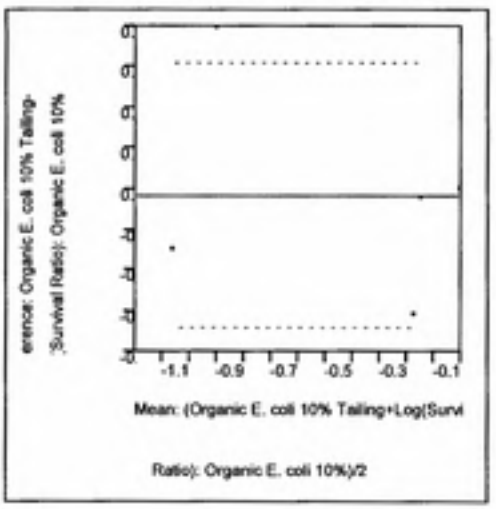
Lower 95%	-0.1195
N	5
Correlation	0.91997

Difference: Organic Bacillus 100% Tailing-Log(Survival Ratio): Organic Bacillus 100%



Organic Bacillus 100% Tailing	-0.2962	t-Ratio	-0.09653
Log(Survival Ratio): Organic Bacillus 100%	-0.2927	DF	4
Mean Difference	-0.0035	Prob > |t|	0.9277
Std Error	0.03667	Prob > t	0.5361
Upper 95%	0.09828	Prob < t	0.4639
Lower 95%	-0.1054		
N	5		
Correlation	0.9375		

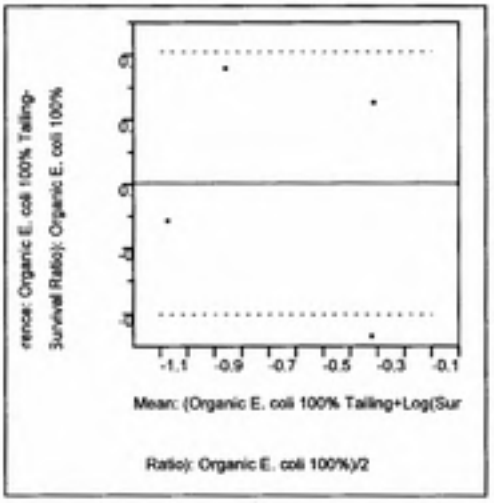
Difference: Organic E. coli 10% Tailing-Log(Survival Ratio): Organic E. coli 10%



Organic E. coli 10% Tailing	-0.465	t-Ratio	-0.15134
Log(Survival Ratio): Organic E. coli 10%	-0.4473	DF	4
Mean Difference	-0.0178	Prob > |t|	0.8870
Std Error	0.11751	Prob > t	0.5565
Upper 95%	0.30847	Prob < t	0.4435
Lower 95%	-0.344		
N	5		

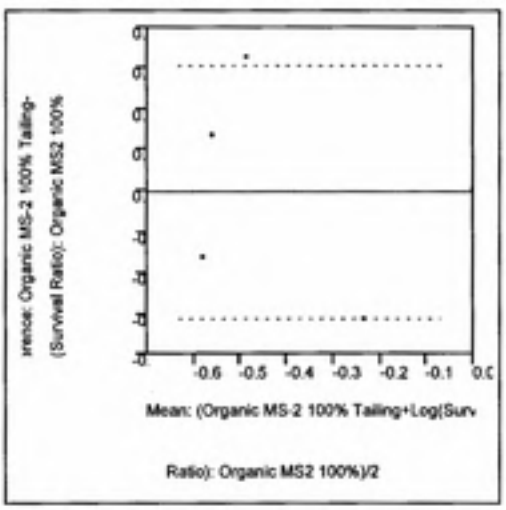
Correlation	0.87805

Difference: Organic E. coli 100% Tailing-Log(Survival Ratio): Organic E. coli 100%



Organic E. coli 100% Tailing	-0.5133	t-Ratio	0.023431
Log(Survival Ratio): Organic E. coli 100%	-0.515	DF	4
Mean Difference	0.00171	Prob > |t|	0.9824
Std Error	0.07303	Prob > t	0.4912
Upper 95%	0.20446	Prob < t	0.5088
Lower 95%	-0.201		
N	5		
Correlation	0.93561		

Difference: Organic MS-2 100% Tailing-Log(Survival Ratio): Organic MS2 100%



Organic MS-2 100% Tailing	-0.3736	t-Ratio	-0.04315
Log(Survival Ratio): Organic MS2 100%	-0.3712	DF	4
Mean Difference	-0.0024	Prob > |t|	0.9677
Std Error	0.05571	Prob > t	0.5162
Upper 95%	0.15228	Prob < t	0.4838
Lower 95%	-0.1571		
N	5		
Correlation	0.89123		

Nonlinear Fit

Response: Synthetic E. coli 100%, Predictor: One-Hit Two-Population Prediction

Control Panel

Converged in Gradient

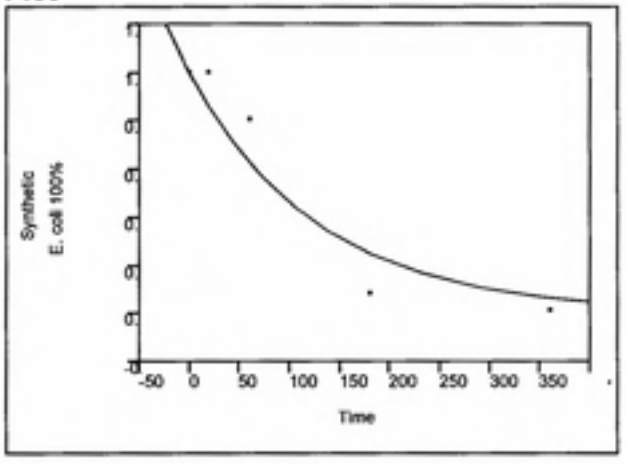
Criterion	Current	Stop Limit
Iteration	7	60
Obj Change	3.231484e-13	1e-15
Relative Gradient	4.7127489e-7	0.000001
Gradient	0.0000137011	0.000001

Parameter	Current Value	Lock
Fraction	0.999	X
Slope	-0.007716877	

SSE
0.0821046363N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Fraction	0.999	0	1
Slope	-0.007716877	-0.0499	-0.0166

Solution

SSE	DFE	MSE	RMSE
0.0821046363	4	0.0205262	0.1432695

Parameter	Estimate	ApproxStdErr
Fraction	0.999	0
Slope	-0.007716877	0.00220389

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Synthetic MS2 100%, Predictor: One-Hit Two-Population Prediction

Control Panel

Converged in Gradient

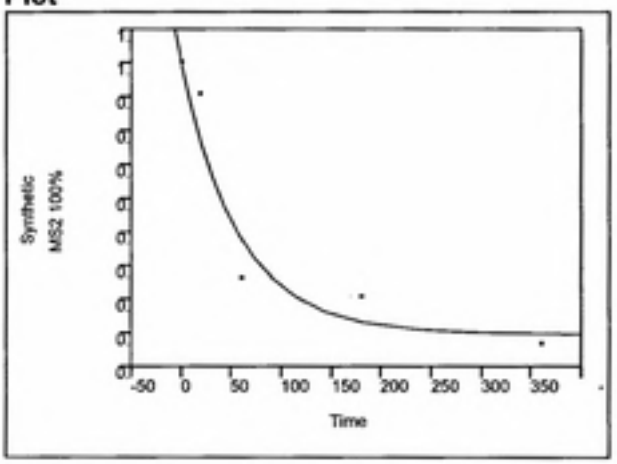
Criterion	Current	Stop Limit
Iteration	3	60
Obj Change	2.505528e-11	1e-15
Relative Gradient	5.6883786e-7	0.000001
Gradient	4.2327693e-6	0.000001

Parameter	Current Value	Lock
Fraction	0.8035000693	
Slope	-0.017353135	

SSE
0.0416065257N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Fraction	0.8035000693	0.44331	1.32994
Slope	-0.017353135	-0.0279	-0.0093

Solution

SSE	DFE	MSE	RMSE
0.0416065257	3	0.0138688	0.117766

Parameter	Estimate	ApproxStdErr
Fraction	0.8035000693	0.09758864
Slope	-0.017353135	0.0070744

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Organic Bacillus 10%, Predictor: One-Hit Two-Population Prediction

Control Panel

Converged in Gradient

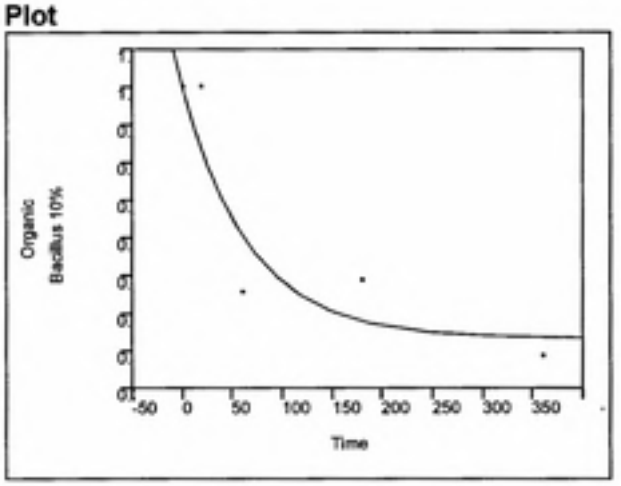
Criterion	Current	Stop Limit
Iteration	5	60
Obj Change	2.703495e-11	1e-15
Relative Gradient	7.7615175e-7	0.000001
Gradient	5.3995792e-6	0.000001

Parameter	Current Value	Lock
Fraction	0.6669184275	
Slope	-0.014838737	

SSE
0.0657834359N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Fraction	0.6669184275	0.61378	1.84134
Slope	-0.014838737	-0.008	-0.0027

Solution

SSE	DFE	MSE	RMSE
0.0657834359	3	0.0219278	0.1480804

Parameter	Estimate	ApproxStdErr
Fraction	0.6669184275	0.13221074
Slope	-0.014838737	0.00951397

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Organic Bacillus 100%, Predictor: One-Hit Two-Population Prediction

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	6	60
Obj Change	4.434792e-12	1e-15
Relative Gradient	9.2996043e-7	0.000001
Gradient	4.7798076e-6	0.000001

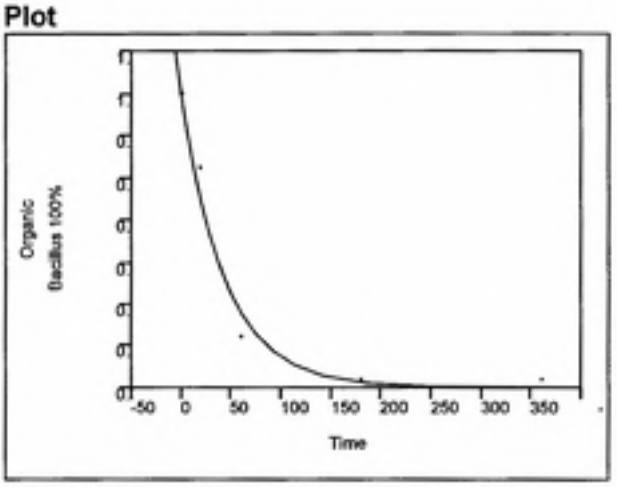
Parameter	Current Value	Lock
Fraction	0.6990468319	
Slope	-0.022623259	

SSE
0.0093007118N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
Fraction	0.6990468319	0.33346	1.00038
Slope	-0.022623259	-0.0223	-0.0074

Solution

SSE	DFE	MSE	RMSE
0.0093007118	3	0.0031002	0.0556798

Parameter	Estimate	ApproxStdErr
Fraction	0.6990468319	0.04211129
Slope	-0.022623259	0.00484493

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Organic E. coli 10%, Predictor: One-Hit Two-Population Prediction

Control Panel

Converged in Gradient

Criterion	Current	Stop Limit
Iteration	6	60
Obj Change	2.588414e-12	1e-15
Relative Gradient	6.7082162e-7	0.000001
Gradient	0.0000216153	0.000001

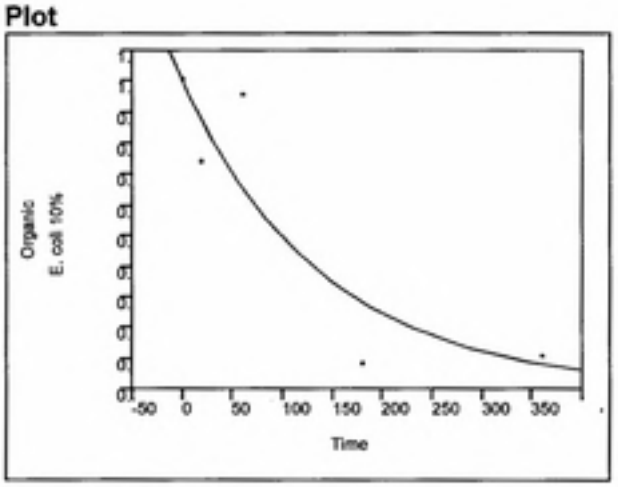
Parameter	Current Value	Lock
Fraction	0.999	X
Slope	-0.007058582	

SSE
0.1488060067N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

.

Plot



Parameter	Estimate	Low	High
Fraction	0.999	0	1
Slope	-0.007058582	-0.0116	-0.0039

Solution

SSE	DFE	MSE	RMSE
0.1488060067	4	0.0372015	0.1928769

Parameter	Estimate	ApproxStdErr
Fraction	0.999	0
Slope	-0.007058582	0.00267696

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Organic E. coli 100%, Predictor: One-Hit Two-Population Prediction

Control Panel

Converged in Gradient

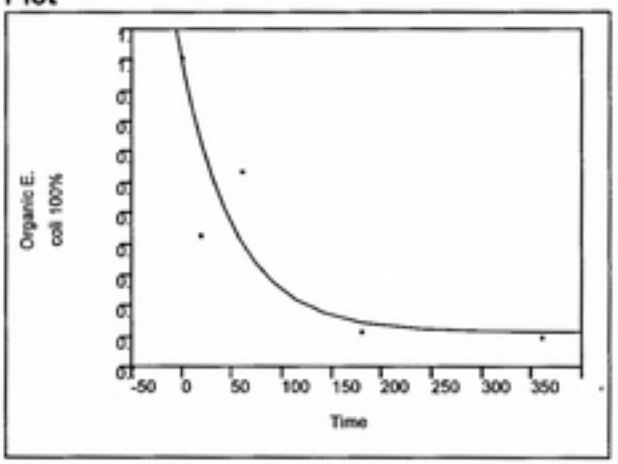
Criterion	Current	Stop Limit
Iteration	14	60
Obj Change	1.033094e-12	1e-15
Relative Gradient	7.4060174e-7	0.000001
Gradient	5.7533541e-6	0.000001

Parameter	Current Value	Lock
Fraction	0.8866253193	
Slope	-0.018580456	

SSE
0.143672196N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Fraction	0.8866253193	0.54886	1.64658
Slope	-0.018580456	-0.0089	-0.003

Solution

SSE	DFE	MSE	RMSE
0.143672196	3	0.0478907	0.2188395

Parameter	Estimate	ApproxStdErr
Fraction	0.8866253193	0.17646205
Slope	-0.018580456	0.01259819

Solved By:
Analytic Gauss-Newton

Nonlinear Fit

Response: Organic MS2 100%, Predictor: One-Hit Two-Population Prediction

Control Panel

Converged in Gradient

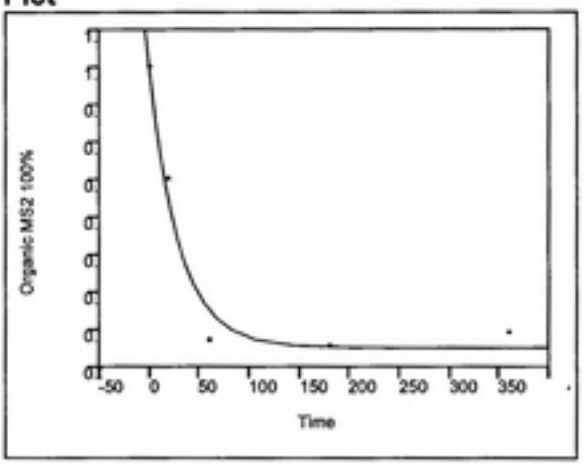
Criterion	Current	Stop Limit
Iteration	10	1000
Obj Change	4.474617e-13	1e-15
Relative Gradient	4.9562611e-7	0.000001
Gradient	1.8172278e-6	0.000001

Parameter	Current Value	Lock
Fraction	0.7490888925	
Slope	-0.033288823	

SSE
0.0122215337N
5

Edit Alpha
0.050Convergence Criterion
0.00001Goal SSE for CL

Plot



Parameter	Estimate	Low	High
Fraction	0.7490888925	0.40175	1.20525
Slope	-0.033288823	-0.026	-0.0087

Solution

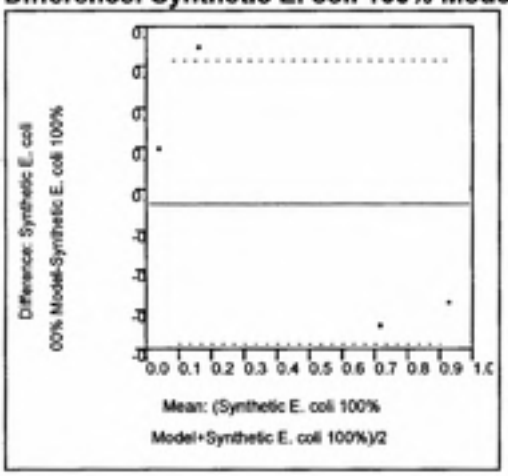
SSE	DFE	MSE	RMSE
0.0122215337	3	0.0040738	0.0638267

Parameter	Estimate	ApproxStdErr
Fraction	0.7490888925	0.04433216
Slope	-0.033288823	0.007785

Solved By:
Analytic Gauss-Newton

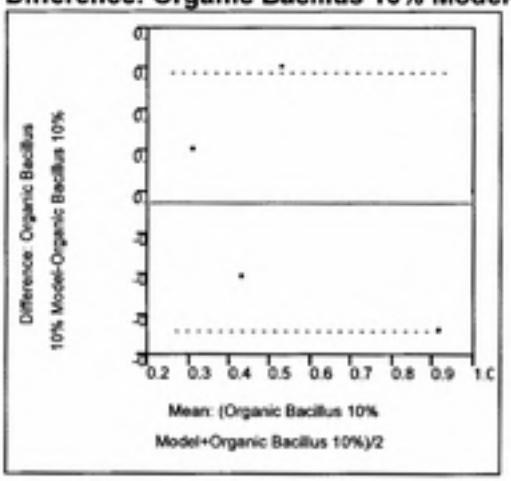
Matched Pairs

Difference: Synthetic E. coli 100% Model-Synthetic E. coli 100%



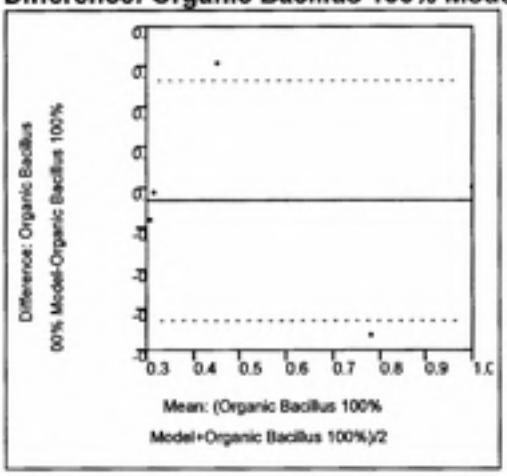
Synthetic E. coli 100% Model	0.56001	t-Ratio	-0.28543
Synthetic E. coli 100%	0.57811	DF	4
Mean Difference	-0.0181	Prob > |t|	0.7895
Std Error	0.06343	Prob > t	0.6053
Upper 95%	0.158	Prob < t	0.3947
Lower 95%	-0.1942		
N	5		
Correlation	0.97273		

Difference: Organic Bacillus 10% Model-Organic Bacillus 10%



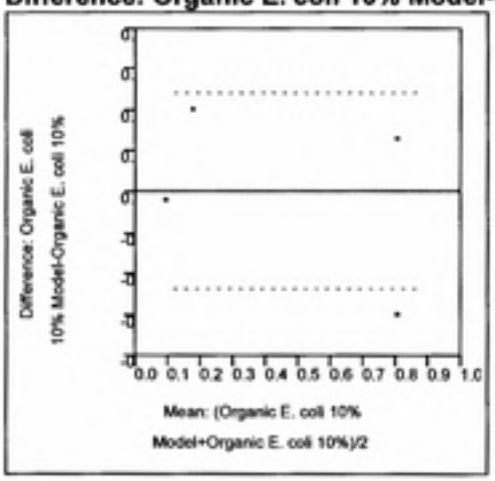
Organic Bacillus 10% Model	0.63022	t-Ratio	-0.25218
Organic Bacillus 10%	0.64457	DF	4
Mean Difference	-0.0143	Prob > |t|	0.8133
Std Error	0.0569	Prob > t	0.5933
Upper 95%	0.14363	Prob < t	0.4067
Lower 95%	-0.1723		
N	5		
Correlation	0.92682		

Difference: Organic Bacillus 100% Model-Organic Bacillus 100%



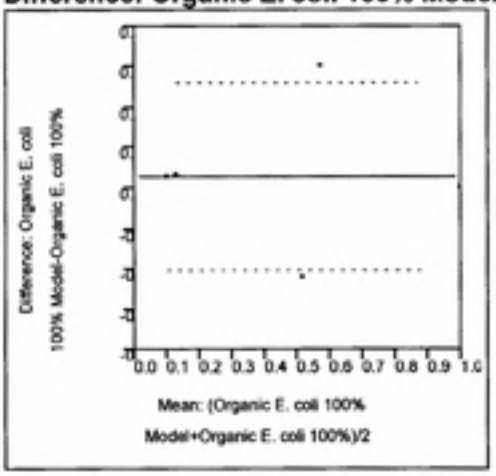
Organic Bacillus 100% Model	0.56809	t-Ratio	-0.28909
Organic Bacillus 100%	0.57426	DF	4
Mean Difference	-0.0062	Prob > |t|	0.7869
Std Error	0.02134	Prob > t	0.6066
Upper 95%	0.05309	Prob < t	0.3934
Lower 95%	-0.0654		
N	5		
Correlation	0.98911		

Difference: Organic E. coli 10% Model-Organic E. coli 10%



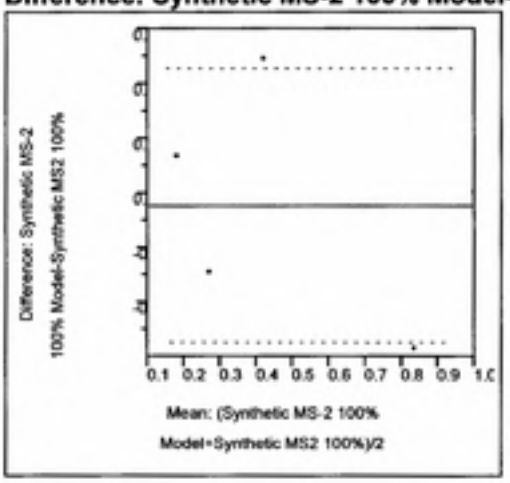
Organic E. coli 10% Model	0.57693	t-Ratio	0.019525
Organic E. coli 10%	0.57525	DF	4
Mean Difference	0.00168	Prob > |t|	0.9854
Std Error	0.08625	Prob > t	0.4927
Upper 95%	0.24116	Prob < t	0.5073
Lower 95%	-0.2378		
N	5		
Correlation	0.90589		

Difference: Organic E. coli 100% Model-Organic E. coli 100%



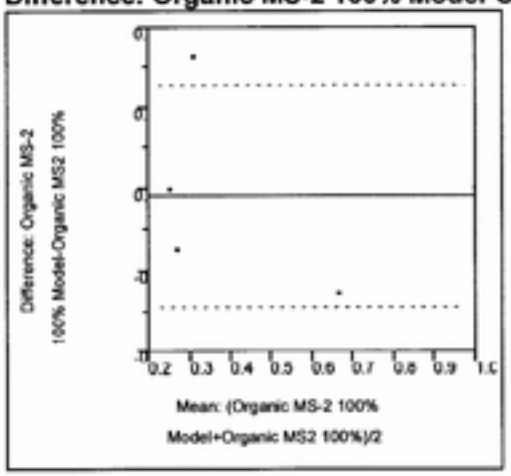
Organic E. coli 100% Model	0.47762	t-Ratio	0.327554
Organic E. coli 100%	0.45022	DF	4
Mean Difference	0.0274	Prob > |t|	0.7597
Std Error	0.08364	Prob > t	0.3798
Upper 95%	0.25962	Prob < t	0.6202
Lower 95%	-0.2048		
N	5		
Correlation	0.87949		

Difference: Synthetic MS-2 100% Model-Synthetic MS2 100%



Synthetic MS-2 100% Model	0.53489	t-Ratio	-0.27045
Synthetic MS2 100%	0.54712	DF	4
Mean Difference	-0.0122	Prob > |t|	0.8002
Std Error	0.0452	Prob > t	0.5999
Upper 95%	0.11327	Prob < t	0.4001
Lower 95%	-0.1377		
N	5		
Correlation	0.96534		

Difference: Organic MS-2 100% Model-Organic MS2 100%



Organic MS-2 100% Model	0.49842	t-Ratio	-0.17944
Organic MS2 100%	0.50284	DF	4
Mean Difference	-0.0044	Prob > |t|	0.8663
Std Error	0.02462	Prob > t	0.5668
Upper 95%	0.06394	Prob < t	0.4332
Lower 95%	-0.0728		
N	5		
Correlation	0.9867		