# Minimizing the spread of New Zealand mud snails through decontamination of fishing gear: A comparison of approaches

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Abstract

New Zealand mud snails (NZMS) are an invasive species recently discovered in economically important trout streams in the Great Lakes region. NZMS are thought to be transported by attaching to fishing equipment. To help control NZMS spread we evaluated the effectiveness of several chemical compounds (Virkon Aquatic, Formula 409, bleach, and water as a control), 2 application methods (soaking and spraying) and two application durations (10 and 20 minutes) at decontaminating fishing waders. Following chemical exposure, we assessed NZMS mortality at timed intervals after placing snails in recovery chambers. One hour after exposure we observed differences in mean NZMS mortality (+/- SD) among chemical treatments, with the greatest caused by Formula 409 (100% +/- 0.0). Virkon resulted in 56.3% +/-23.94, bleach resulted in 68.8% +/- 23.94, and water had no effect on NMZS mortality. Neither application method nor duration had a significant effect on mortality. These results show that Formula 409, a readily available household cleaning product, holds promise as a practical way to decontaminate fishing gear, and minimize the spread of NZMS in aquatic ecosystems.

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## Introduction

New Zealand mud snails (NZMS), *Potamopyrgus antipodarum* (Figure 1), are native to the lakes of New Zealand and adjacent small islands (Benson et al., 2018). These snails inhabit rivers, streams, vegetations, mud, gravel, and woody debris (New Zealand mud snail Management and Control Plan Working Group, 2007). They successfully invaded water bodies in Europe, Asia, Australia, and North America (Benson et al., 2018), and were first discovered in 1987 in United States in Idaho in the waters of the Snake River (Stout et al., 2016). Since then, NZMS have spread across the American West. In the Great Lakes Region, NZMS have been detected in the Great Lakes proper since 1991, with river populations discovered in the state of Wisconsin in 2012 and recently in Michigan in 2015 in the Pere Marquette and Au Sable River system (Michigan, USA). NZMS continue to rapidly spread throughout the country with the most recent range expansion occurring the Great Lakes Region.

Identifying the spread pathways is an important step towards reducing and preventing invasion to uninfected water bodies (U.S. Fish and Wildlife Service/Fish and Aquatic Conservation, 2015). Invasive species are spread by ballast water exchange, adhesion to ships, and fishing equipment. NZMS are easily transported by attaching to recreational gear such as fishing waders or boots (Stockton, 2013). Researchers hypothesize that ballast water is the primary pathway of NZMS transmission, while contaminated fishing waders are the secondary source of transport (Hosea & Finlayson, 2005; State of Michigan, 2018).

Invasive species can have critical impacts on ecosystems because they affect native species through competition, predation, disease transfer, and habitat alteration (Ellender, Woodford, Weyl, & Cowx, 2014). For example, NZMS negatively affect fish populations by reducing the nutrition fish obtain from their diet (Stout, Avila, & Fetherman, 2016). NZMS are primarily grazers, and feed on periphytic algae, animals and plants waste, as well as sediments (Benson et al., 2018). NZMS may impact food webs and the function of ecosystems by dominating nutrient cycling (Schisler, Vielra, & Walker, 2008) and consuming large amounts of algae (Hall, Tank, & Dybdahl, 2003). North American trout fisheries are negatively affected by NZMS (Vinson, & Baker, 2008). For example, a study showed that rainbow trout which fed on NZMS lost 0.14–0.48 % of their initial body weight per day when they consumed NZMS due to the lack of nutrients in NZMS (Vinson, & Baker, 2008). The shell and operculum of NZMS protects the snail from predators and harsh conditions; it also allow them to sometimes pass through the digestive system of trout and still remain viable (Kabat and Hershler, 1993, State of Michigan, 2018). This in turn is believed to make the fish feel full without having eaten food of high nutritional value, and as a result, they lose weight (State of Michigan, 2018).

To prevent spread of NZMS into unaffected water bodies, effective decontamination methods need to be developed. Previous research has evaluated the decontaminant Virkon® Aquatic and evaluated different methods of application (soaking and spraying) and durations (Stockton et al., 2013). Virkon® Aquatic is a powder when mixed with water forms a strong disinfectant to treat fish pathogens (Syndel USA, 2016). Virkon® Aquatic can be prepared in different concentration when diluted (e.g., 0.5%, 1%, or 2%). Concentrations can be verified using Virkon® test strips. Virkon® Solution will gradually loose its activity over time; its ingredients are active only 7-14 days after mixing (Syndel USA, 2016). Studies showed a 100% mortality rate when waders were soaked for 30 minutes with 20 g/L Virkon® Aquatic (Stockton et al., 2013). Soaking the snails for 15 and 20 minutes in 20 g/L of Virkon® Aquatic solution resulted of 99% mortality (Stockton et al., 2013). Soaking NZMS in 10 g/L of Virkon® Aquatic solution yielded 90% mortality after being soaked for 15 minutes, and 97% mortality after being soaked in 30 minutes (Stockton et al., 2013). Researchers also tested spraying applications that varied in concentration and found that 99% of NZMS were killed 30-40 minutes before NZMS were rinsed from the 20 g/L Virkon® Aquatic solution (Stockton et al., 2013). While Virkon® Aquatic is an effective control measure, we hypothesize consumers are less likely to purchase it because of the careful handling procedures it requires. It is rarely available in stores, and requires dilution and the use of test strips to ensure effectiveness. The necessity of users to prepare the solution and the fairly short duration after which the solution remains effective, are factors that probably deter fisherman from using Virkon, instead using similar products that are pre-mixed and ready to use.

Previous studies have shown that Sparquat 256 was effective in achieving a 100% NZMS mortality, however, the manufacture of this product has been discontinued. Sparquat 256 is a germicidal cleaner produced by Spartan Chemical Company, Inc., Maumee, Ohio (Kegley, Hill, Orme, & Choi, 2016). Sparquat contains 12.5% of a Quaternary Ammonium Compound (QAC) called Alkyl (40% $C_{12}$,50% $C_{14}$, 10% $C_{16}$) Dimethylbenzyl Ammonium Chloride (Department of Natural Resource, 2015). QAC is an EPA-registered disinfectant cleaning product and is toxic to invertebrates (The Clorox Company, 2018). Schisler and colleagues achieved a 100% mortality of NZMS when they were soaked in 3.1% Sparquat 256 for 10 minutes (2008). Sparquat 256's discontinuation has evoked studies to find another chemical that is equally effective to replace it. QAC is also found in other disinfectant products. Formula 409 Multi-Purpose Cleaner is a cleaning product made by The Clorox Company, Oakland, California (The Clorox Company, 2018). It contains 0.3% of Alkyl (40% $C_{12}$,50% $C_{14}$, 10% $C_{16}$) Dimethylbenzyl Ammonium

Chloride (Wisconsin Department of Natural Resources, 2018). Formula 409 contains Ethanolamine which is known to have degreasing properties; this helps to loosen the snails' opercular and allow for better exposure of the chemical (Schisler et al., 2008; The Clorox Company, 2018). Schisler and fellow researchers found that 50% dilution Formula 409 was not sufficient in killing NZMS, while a 10-minute submersion of Formula 409 resulted in 100% mortality of NZMS (2008). While Sparquat 256 is discontinued, Formula 409 may provide a safe and affordable way to decontaminate and help slow the spread of NZMS. It is a readily available household cleaning product which appears to be a practical way to decontaminate fishing gear and minimize the spread of NZMS in aquatic ecosystems.

A potentially effective approach to minimize NZMS transport is to disinfect fishing equipment. However, results from past research are still unclear and inconsistent to the public as to what chemical treatments and application methods (e.g., soaking vs. spraying) are most effective at causing mortality and likely to be used in preventing the spread of NZMS. Different concentration levels of various products presented confusing results to the readers. To test the effectiveness of common decontamination chemicals on NZMS, a laboratory experiment was conducted which tested three different chemical decontamination agents. The chemicals used are Virkon® Aquatic, Formula 409 Multi-Purpose Cleaner, household bleach, and water as a control (Figure 2). The effectiveness of these chemicals was measured by treating snails with the chemicals, different application techniques (i.e., soaking and spraying) and for different durations, and assessing snail mortality. Our study evaluated decontamination procedures to determine a decontamination procedure that maximizes mortality to NZMS that attach to fishing gear. The research questions addressed in this paper are: Which disinfectant is most effective at causing NZMS mortality? Does the method of application (i.e., soaking vs. spraying) influence

NZMS mortality? And does time of exposure (10 vs. 20 minutes) affect mortality? We were able to answer these questions by testing the effects of three chemical treatments. Formula 409 Multi-Purpose Cleaner, Virkon® Aquatic, and household bleach. With this approach we hope to improve understanding of how to best prevent the spread of NZMS via wading gear decontamination

**Methods and Materials.**

NZMS were collected in mid-summer of 2017 from the Au Sable River, a major tributary to Lake Huron. Several hundred NZMS were collected near the town of Grayling and placed into a 10-gallon holding tank with clean tap water held at room temperature. Native rock and organic matter (e.g., leaves) were placed into the holding tank to provide food and help mimic natural conditions for the snails. Prior to the experiment, a bucket was filled with tap water that then sat for 48 hours to allow the water to reach room temperature and off gassing of chlorine.

On the day of the experiment, 64 adult snails were randomly selected from the holding tank and placed into a 500-millimeter glass beaker filled with water to prepare for the experiment. Snail sizes were measured with a ruler to the nearest millimeters along the longest axis of the snail shell. Mean (+/- SD) snail length was 45.9 mm (+/- 0.496). After measuring length, snails were placed in a petri dish with tap water to assess their viability before treatment exposure. Movement was used to determine if the snails were alive. Afterwards, snails were placed into a 1000 mL beaker filled with tap water for holding until experiment begins. A temperature logger was used to record room temperature and water temperature was measured using a thermometer. The pH of tap water was measured using pH paper.

The experimental design was a fully crossed 4 x 2 x 2 factorial (3 chemical treatments and one control treatment × 2 exposure durations × 2 application types). We diluted 0.70 ounces Virkon® Aquatic in 32 quarts of water to reach 2% concentration equivalent to the manufacturer's label recommendation. Virkon ® test strips were used to confirm the desired concentration. We also diluted 5.25% sodium hypochlorite in 32 quarts of water to obtain a 10% household bleach solution, the typical recommended concentration for household bleach by OSHA and CDC (Center for Disease Control and Prevention, 2016). Formula 409 Multi-Purpose Cleaner was used at its pre-made 100% concentration. We transferred the chemicals into identical spray bottles to standardize spray volume. The spray bottles containing chemicals requiring dilution (Virkon® Aquatic and household bleach) were then filled with water to achieve the target concentrations. Formula 409 Multi-Purpose Cleaner was transferred from the bottle in which it was purchased to the standardized test spray bottle. An additional set of spray bottles containing tap water were prepared for rinsing the chemicals from the snails.

Because NZMS cling to wading material, which is a common means for spread, wader fabric was cut to the size of experimental chambers (60 x15 mm polystyrene disposable petri dishes) (Figure 3). The waders used in this experiment were Cabela's Men's Premium Breathable Stockingfoot Fishing Waders with 4MOST DRY-PLUS®. The wader material in each petri dish was sprayed with water immediately prior to the start of the experiment to simulate their condition after a fisherperson exits the water. NZMS were placed directly onto the wader material in each petri dish.

Prior to spraying each snail, each spray bottle was primed by discharging two test sprays away from the petri dishes to achieve a full spray (approximately 1mL). Then, snails were placed on the wader material. All petri dishes were sprayed with the appropriate chemical and ordered randomly in the working area. The soaking application involved filling petri dishes with each decontaminant to a level that ensured snails would be fully submerged by each solution. NZMS were left exposed to each treatment for either 10 or 20 mins. Thereafter, snails were picked up with forceps, rinsed with water for 5 seconds, and placed in recovery chamber (100 x15 mm polystyrene disposable petri dish) that contained sufficient water to submerge each snail (Figure 4). Then snails were placed in the recovery area that was separated from the working area to prevent contamination. To assess for movement, a mark was made on the underside of each recovery chamber to indicate the location of the snail. Snails in their petri dishes were then placed randomly in the recovery area on the table. An immediate viability assessment was made, followed by assessment 1, 6, 24, 48, 72, and 96 hours. After each assessment, a new mark was placed on the petri dish to track if the snail moved again. With these methods, we assessed the viability of each snail, which was deemed to be dead after the cessation of movement

After the experiment was performed, mean percent mortality for each treatment were compared using 2-way analysis of variance (ANOVA) to test for differences in chemical treatment, application technique (spray and soak), and exposure duration (10 minutes and 20 minutes). Post-hoc Tukey HSD (Honestly Significant Difference) test was calculated to determine significant differences among groups. Survival analysis was conducted using Kaplan-Meier estimates. Survival function curves were compared using pairwise log-rank test after Bonferonni corrections.

**Results**

NZMS mortality differed among decontaminants (ANOVA, $P < 0.001$) (Figure 5, Table 2). The greatest mean NZMS mortality (+/- SE) 1 hour after exposure to the decontaminants across all applications and durations was caused by Formula 409: 100% (+/- 0). Bleach resulted in a mortality (+/- SE) of 68.75% (+/- 11.97) one hour after exposure (Figure 5). Virkon® Aquatic had a mean mortality of 56.25% (+/- 11.97) one hour after exposure. Water had a mean mortality of 0% (+/- 0). Neither exposure duration (P = 0.48) nor application method (P = 0.16) had an effect on NZMS mortality (Figure 6 and 7), nor were there any significant interactions in the fully crossed ANOVA model (P>0.23) (Table 3).

Kaplan-Meier analysis showed a 6.3% survival probability after 1 hour for NZMS treated with Formula 409, across all applications and exposure durations (Figure 8). After 24 hours, survival probability was 0% (Figure 8). NZMS treated with household bleach had an 81.2% probability of survival after 1 hour and 62.5% after the final 96-hour viability assessment (Figure 8). NZMS treated with Virkon Aquatic had a 37.5% survival probability after 1 hour and 18.8% after 96 hours (Figure 8). NZMS treated with the control (i.e., water) had a 100% survival probability throughout the entire viability assessment duration. Significant differences of survivorship curves were observed between household bleach and Formula 409 (P<0.001), household bleach and Virkon Aquatic (P=0.034), household bleach and water (P=0.044), Virkon and Water (P<0.001), Formula 409 and water (P<0.001) (Table1).

## Discussion

Our study tested chemical decontaminants widely used for bacteria and pathogens, however, previous published literature has tested some of them for NZMS spread prevention with contrasting results. Our aim was to perform a more straightforward assessment on the efficacy of these three chemicals and their effectiveness at causing NZMS mortality. Our results differ then previous studies, in that Virkon® aquatic, a leading chemical agent for disinfection and advocated for by many natural resources agencies, proved to be less effective than the easily attainable household cleaner Formula 409. Stockton showed that bathing NZMS in 10 g/L of

Virkon® aquatic resulted in 100% mortality after 20 minutes of chemical exposure (2013). Our results differ from Stockton's experiment. Because of this and the multi-step process to use it (i.e., purchase online, mix to concentration, etc.), Formula 409 seems to be a better chemical agent and more easily accessible.

A mortality rate of 100% was expected when treating NZMS with Formula 409. A higher mortality rate for snails treated for 20 minutes in comparison to the ones treated for only 10 minutes was expected. Also, a higher mortality rate with NZMS that were soaking in the chemical in comparison to the snails sprayed only. Formula 409 resulted in 100% mortality, which makes it a feasible decontaminant for NZMS given the effectiveness and ease of obtaining and using. Meaning, all one needs to do is go to a convenient store, purchase the bottle and use at the already prepared concentration right out of the bottle. It is portable and relatively inexpensive.

Household bleach, another commonly used disinfectant proved to be ineffective at causing mortality to NZMS. The expected results were uncertain with household bleach treatment using the recommended concentration by OSHA and the CDC. We expected a higher mortality rate for snails treated for 20 minutes in comparison to the ones treated for only 10 minutes. We also expected a higher mortality rate with NZMS that were soaking in the chemical in comparison to the snails sprayed only. Our results indicate that household bleach, when used for NZMS decontamination resulted in only 68.75% (+/- 11.97) mortality one hour after exposure. Some snails treated with household bleach did not die, probably an increase in the concentration of household bleach in future research might increase the mortality rate. Researchers found that undiluted household bleach caused damage to fabric of waders (Hosea & Finlayson, 2005). It changed the color, reduced flexibility (stiffening), cracked neoprene, and teared the fabric on the boots area (Hosea & Finlayson, 2005). Although we did not test for material damage, we have been applying 409 to our waders and gear heavily for extended periods of time and have noticed no damage.

We expected high mortality with Virkon® aquatic due to its widespread use and it being a popular decontaminant used by agencies and aquaculture facilities. We anticipated a higher mortality rate for snails treated for 20 minutes in comparison to the ones treated for only 10 minutes. Also, a higher mortality rate was expected with NZMS that were soaking in the chemical in comparison to the snails sprayed only. Surprisingly, Virkon® Aquatic resulted in lower mortality rates than expected, this has implications as it is a widely used disinfectant and commonly used with natural resources agencies. Virkon® Aquatic, according to manufacturer's label is effective for killing a variety of pathogens and diseases, but our results suggest it is less effective at killing NZMS.

Overall, Formula 409 proved to be the most effective at inducing mortality to NZMS, across all application types and exposure durations. These results suggest that Formula 409 is an effective and efficient means to decontaminate fishing and wading gear. Formula 409 a readily available household disinfectant holds promise to help prevent spread of NZMS on fishing gear. No significant difference between spraying and soaking, nor duration 10 mins and 20 mins was found, for all chemicals.

Future research will couple this data with surveys responses from anglers on the likelihood they will use a particular decontamination protocol. The result will be used to develop best management approaches that maximize mortality of NZMS and help prevent NZMS spread.

**Conclusion**

The research conducted could be used to help find a way to minimize NZMS population and help the ecosystem to go back to its balance. The research could help fishermen to save the fish by finding a way to decontaminate their waders and fishing gear from NZMS. The conducted study is important especially for Michigan because we are inhabitants of the Great Lakes region. Our research suggests that the widely available, relatively cheap, mobile chemical, Formula 409 is the best in killing NZMS. Whereas with Virkon® Aquatic, consumers have to mix and store. Also, Virkon® Aquatic is only available online. Household bleach is not as effective because over time it becomes less effective due to dilution/off gassing. From these results, we recommend using Formula 409 at 100% strength for decontaminating waders and other fishing gear (e.g., nets, boots, etc.). Soaking waders and fishing gear are a time-consuming process, and fishermen would prefer to utilize a different method to disinfect their gear while maintaining the quality of fishing gear.

## Appendix

In order to minimize the spread of invasive species, planning before, during, and after going to the field is essential. The Wisconsin Department of Natural Resources (WDNR) outlined recommended management practices specific to each surface desired to be disinfected. Before going in the field, it is crucial to be aware of what water bodies are infected with what species, consider dedicating some of working gears to those infected waters, and arrange sampling plans from areas with low invasive species density to the higher ones. While in the water, it is recommended to preform experiments from upstream to downstream and from areas with less vegetation to a higher density one. It is also suggested to frequently clean and check gear while working. Some general practices when done working in the field are to inspect and clean working gear and scrub them with a stiff-bristled brush to remove mud and organic debris. Next, it is suggested to spray liberally with 409 and let sit for a recommended 20 minutes. It is recommended to move away from, the stream when spraying 409, to avoid getting the chemical into the water.


**Figure 1.** New Zealand mud snails placed on a metric ruler for scale (Benson et al., 2018).


**Figure 2.** Common disinfectants used to decontaminate fishing gear. Clorox Bleach, Formula 409 and Virkon® Aquatic. Tap water served as a control.


**Figure 3.** Randomized arrangement of experimental chambers with wader material


**Figure 4.** Recovery chamber containing a single NZMS

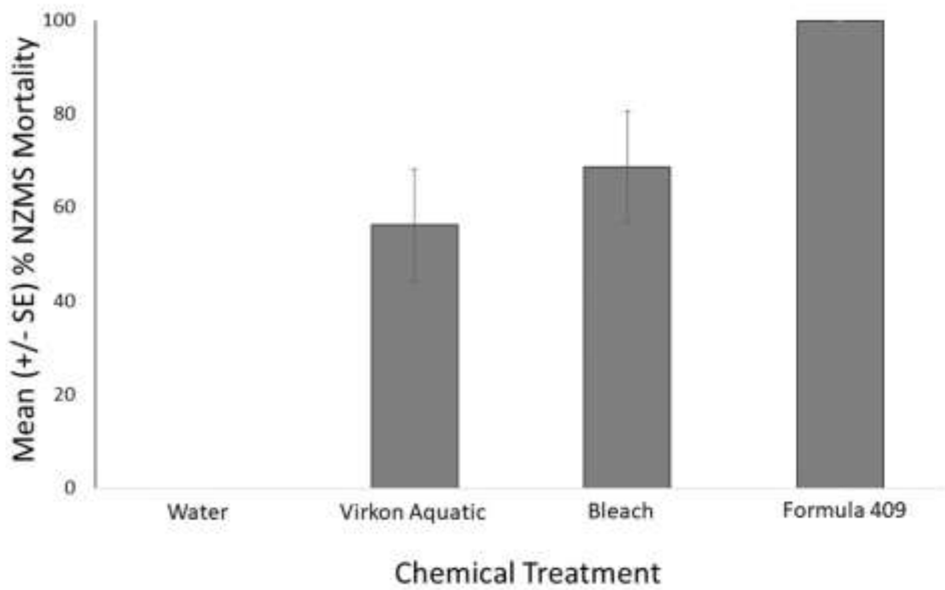


**Figure 5.** NZMS mortality across all treatment combinations. Post-hoc analysis indicates a significant difference on NZMS mortality between Formula 409 and Virkon® Aquatic (P = 0.005). The effect of each decontaminant was different from water (P < 0.001).

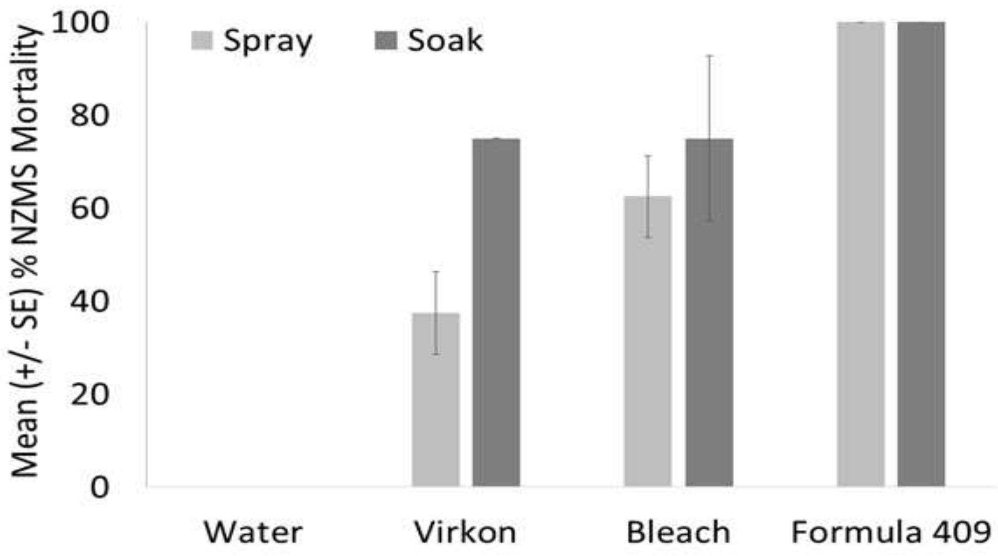

**Figure 6.** Results of the effectiveness of tested chemicals on NZMS mortality and application technique after 1-hour viability assessment. No significant differences were found between spraying and soaking.

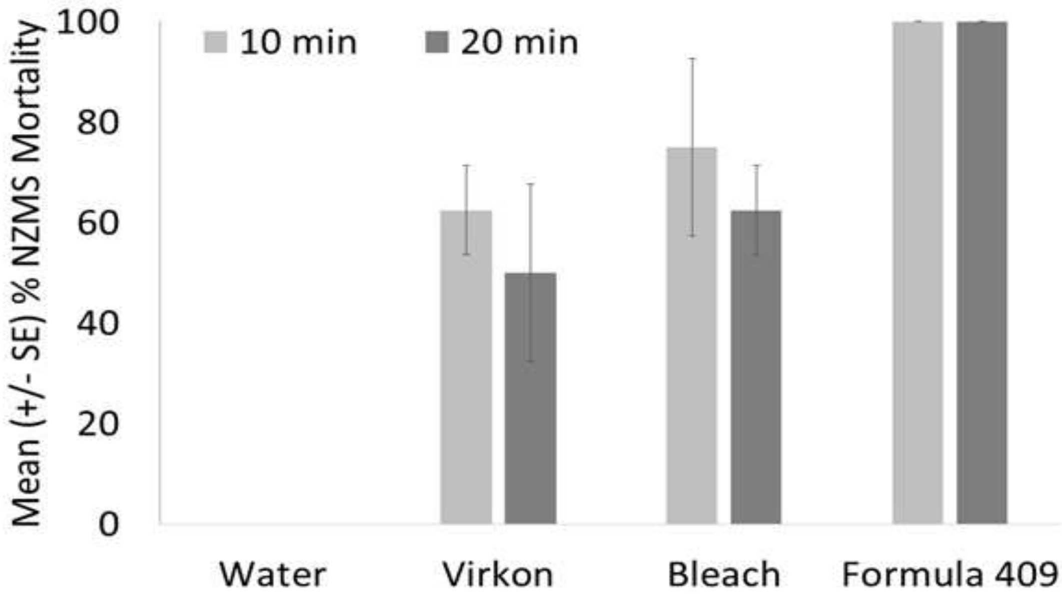


**Figure 7.** Results of the effectiveness of tested chemicals on NZMS mortality and duration of exposure after 1-hour viability assessment. No significant differences were found between 10- and 20-minutes exposure durations.

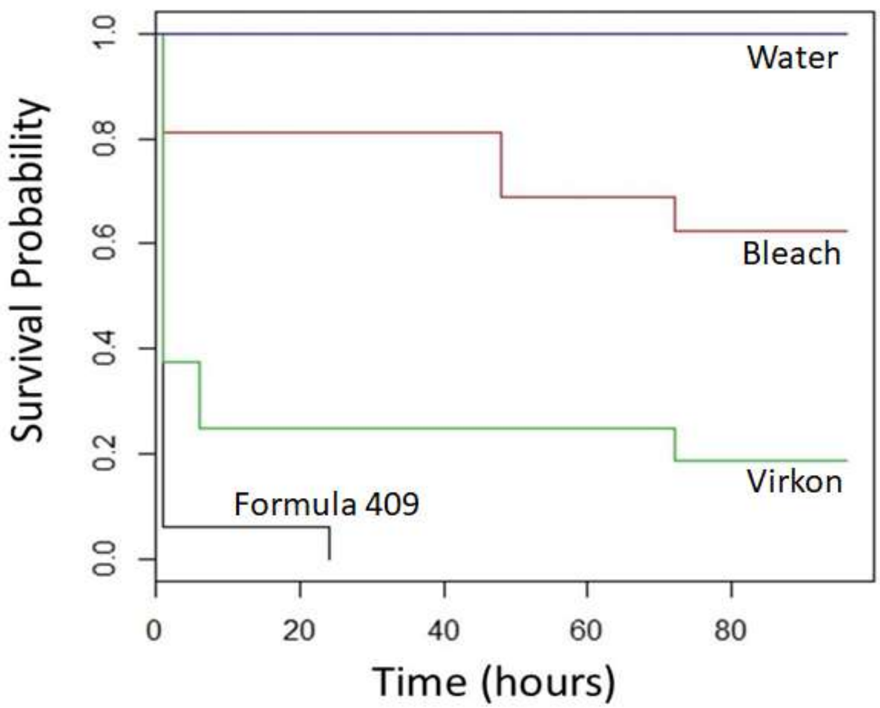


**Figure 8.** Kaplan-Meier survivorship curves for all chemical treatments. Probability of NZMS survival when treated with Formula 409 was 6% at hour 1 and 0% at hour 24.

**Table 1.** Differences in survivorship functions after 96-hour viability assessments. All chemical treatments differed, except for Formula 409 and Virkon® Aquatic (significance accepted at ≤ 0.05).

| | Formula 409 | Bleach | Virkon® Aquatic |
|---|---|---|---|
| Bleach | <0.001* | - | - |
| Virkon® Aquatic | 0.142 | 0.034* | - |
| Water | <0.001* | 0.044* | <0.001* |

**Table 2.** Results summary of NZMS decontamination trials. Values shown are mean percent mortality for each chemical treatment, application type, and exposure duration after 1 hour after

treatment exposure; and the mean (+/- SD) time of apparent mortality throughout the viability assessment duration (96 hours).

| Chemical treatment | Application | Exposure duration (min) | Percent mortality (+/- SD) (after 1 hour ) | Mean (+/-SD) time in hours to mortality |
|---|---|---|---|---|
| Water | Spray | 10 | 0 (0) | >96 (0) |
| | Soak | 10 | 0 (0) | >96 (0) |
| | Spray | 20 | 0 (0) | >98 (0) |
| | Soak | 20 | 0 (0) | >96 (0) |
| Bleach | Spray | 10 | 50 (0.57) | >96 (0) |
| | Soak | 10 | 100 (0) | 60 (45.96) |
| | Spray | 20 | 75 (0.5) | 66 (45.43) |
| | Soak | 20 | 50 (0.57) | 60 (45.96) |
| Virkon Aquatic | Spray | 10 | 50 (0.57) | 25.5 (47.09) |
| | Soak | 10 | 25 (0.5) | 48 (43.82) |
| | Spray | 20 | 75 (0.5) | 2 (2.71) |
| | Soak | 20 | 75 (0.5) | 0.25 (0.5) |
| Formula 409 | Spray | 10 | 100 (0) | 0 (0) |
| | Soak | 10 | 100 (0) | 6 (12) |
| | Spray | 20 | 100 (0) | 0 (0) |
| | Soak | 20 | 100 (0) | 0 (0) |

**Table 3.** Two-way analysis of variance (ANOVA) to test for differences in chemical treatment, application technique (spray and soak), and exposure duration (10 minutes and 20 minutes).

| | Degree of freedom | Sum Square | Mean Square | F-value | Pr (>F) |
|---|---|---|---|---|---|
| Chemical | 3 | 8.375 | 2.7917 | 22.333 | 3.4e-09 *** |
| Duration | 1 | 0.063 | 0.0625 | 0.500 | 0.483 |
| Application | 1 | 0.250 | 0.2500 | 2.000 | 0.164 |
| Chemical: Duration | 3 | 0.063 | 0.0208 | 0.167 | 0.918 |
| Chemical: Application | 3 | 0.375 | 0.1250 | 1.000 | 0.401 |
| Duration: Application | 1 | 0.063 | 0.0625 | 0.500 | 0.483 |
| Chemical: Duration: Application | 3 | 0.562 | 0.1875 | 1.500 | 0.226 |

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