CONTROL OF *LISTERIA SPECIES* AND OTHER BACTERIA IN CRAWFISH AND CRAB PROCESSING FACILITIES, UTILIZING COPPER DRAINS, COATINGS AND CONCRETE, CONTAINING COPPER IONS

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Dedicated to My Daughter,

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TABLE OF CONTENTS

LIST OF TABLES

ABSTRACT

The antibacterial properties of copper ions against *Listeria spp.*, *Pseudomonas spp*., *Escherichia coli* and other coliforms, and total aerobic bacteria have been investigated in seafood processing environments. Our hypothesis was that drains fabricated from copper and coatings and concrete containing copper ions that have been used for sealing floors and walls could be utilized in crawfish and crab processing facilities as an effective means of controlling *Listeria spp.* and other unwanted bacteria. Copper in the form of copper sulfate pentahydrate was incorporated into coatings and concrete at the salt concentration of 25 ppm (6.36 ppm Cu^{++}). Sampling sites were selected in multiple areas of the processing facilities. Sampling was performed in the course of two-month seafood production period. Bacterial counts were determined by using microbiological selective media. In addition, PCR analysis was applied to detect the presence of *Listeria monocytogenes* in the environmental samples. Copper drains were found to be quite effective against some of the tested bacteria. The counts of *Listeria spp*. and total aerobic bacteria were over one log CFU/cm^2 lower on the copper drains than those detected on the control sites. Neither copper coatings nor copper concrete exerted antimicrobial activity against any microorganisms tested. Additionally, almost all PCR samples were negative for the presence of *Listeria monocytogenes*, suggesting that black colonies grown on Oxford media represented the other species of *Listeria* family. Based on the overall results, the copper-fabricated drains could be used in the seafood processing facilities as one of other approaches for reducing environmental contamination by foodborne pathogens. Regarding the application of copper coatings and concrete at the seafood production plants, future research should be conducted to find the most effective bactericidal copper forms and, subsequently, their effective minimal inhibitory concentrations.

INTRODUCTION

For the last two and a half decades *Listeria monocytogenes* (hereafter referred to as *L. monocytogenes*) has created real challenges to the food industry not only in the United States but worldwide (Rorvik and Yndestad, 1991; Ababouch, 2000; Farber, 2000; Krasovsky et al., 2000; Lindqvist and Westoo, 2000; WHO, 2000; CDC, 2001; Siegman-Igra et al., 2002; Okutani et al., 2004; Vitas et al., 2004). The outbreaks of listeriosis, a highly lethal disease caused by *L. monocytogenes*, which commonly occurs as a result of food consumption and its high lethality forced the USA regulatory agencies to develop and implement a number of regulations and rules designed to reduce the risk associated with *L. monocytogenes* (USDA, 9 CFR 430, 2003). Although *L. monocytogenes* has been found in a great variety of food, the highest threat of this pathogen is in those food products that have a long refrigerated shelf-life and that are generally consumed with little or no heating (Bremer et al., 2003). Among these high-risk products is ready-to-eat seafood (Eklund et al., 1995; Huss et al., 2000; Rocourt et al., 2003; Reij and Antrekker, 2004).

Crawfish and crab production is a multimillion dollar-a-year sector of the Louisiana seafood industry. In 2000-2001 the production of farm-raised crawfish in Louisiana state was estimated at 37 million pounds and with crawfish acreage being 84,635 acres (Louisiana State University Agricultural Center, 2002). Bacterial contamination of ready-to-eat seafood products in the seafood processing environment mainly occurs during post-cooking processes, peeling and packaging (Beresford et al., 2001; Bagge-Ravn et al., 2003; Hoffman et al., 2003; Reij and Antrekker, 2004).

Some strains of *L. monocytogenes* become resident microflora by establishing their sites in drains, wall or floor cracks in processing areas. Despite regular clean-up and sanitation

procedures, this pathogen is shown to persist for many months or even years by means of protective mechanisms such as biofilm formation, temperature and pH resistance (Chavant et al., 2002; Tompkin, 2002). Some studies show that the strains of the established microflora might contaminate final food products and that there is a direct relationship between the environmental contamination level of *L. monocytogenes* and its bacterial level in the final ready-to-eat seafood products (Rorvik et al., 1997; Hoffman et al., 2003; Beresford et al., 2001). Therefore, reducing the general bacterial contamination of the seafood processing environment, especially of post-processing rooms, will lead to a certain decrease in ready-to-eat product contamination by *L. monocytogenes*.

In this research the antimicrobial effect of copper ions against *L. monocytogenes* and other pathogens (*Pseudomonas spp*., *Escherichia coli* and other coliforms) was investigated in seafood processing facilities. Copper was applied in the fabrication of drains, concrete and coatings for the food-processing industry to potentially increase control of the foodborne pathogens, particularly *L. monocytogenes*. Research has shown that environmental areas of seafood processing facilities harbor *Listeria spp.*, thus allowing the bacterial population not just to survive but rather remain persistent for a relatively long period of time (Thimothe et al., 2002; Tompkin, 2002; Lappi, Thimothe et al., 2004). Although copper is known for bacteriostatic and bactericidal properties, it has not been widely and extensively utilized in seafood processing facilities as an effective means of controlling the environmental source of *L. monocytogenes*.

This work is the continuation of the research conducted by Abushelaibi (2005). Although, under laboratory conditions the researcher has already shown the effectiveness of copper ions using copper sheets as well as copper sulfate pentahydrate or copper oxide in the polyutherane coatings against serotype 1/2a of *L. monocytogenes*, the purpose of this study was

to demonstrate the efficacy of copper ions against *Listeria spp.*, particularly *L. monocytogenes* and other bacteria such as *Pseudomonas spp.*, *E. coli* and other coliforms, and other aerobic bacteria in the environmental areas of the seafood processing facilities. In order to test this hypothesis, the efficacy of drain covers fabricated from copper and the antimicrobial effect of coatings and concrete, containing copper ions were tested within a two-month seafood production period at one crab and two crawfish processing plants.

REVIEW OF LITERATURE

L. monocytogenes is a gram-positive, rod-shaped, non-sporeforming, facultative anaerobe that can grow within low temperature parameters. *L. monocytogenes* has been intensively studied for more than half a century. This bacterium belongs to the genus *Listeria* which consists of the other five species: *L. innocua*, *L. ivanovii*, *L. welshimeri*, *L. seeligeri*, and *L. gray* (Rocourt, 1999). Of these six species *L. monocytogenes* is of the greatest concern in terms of public health due to the ability of some of its strains to cause listeriosis in immunocopromised individuals (e.g., AIDS patients, pregnant women, newborns, the elderly, etc) as well as to the ubiquitous nature of this microorganism (Hitchins and Whiting, 2001).

Listeriosis outbreaks have been regularly reported in various parts of the world by international organizations and national agencies of both developed and developing countries. The incidence rate of this disease varies from one region to another. For instance, in Japan the rate of listeriosis that occurred for the past forty years was much lower than that of North America and Western Europe. Annually, as low as 0.65 case occurred per one million people, whereas 5 cases of listeriosis were reported each year in the United States (CDC, 2001; Okutani et al., 2004). The other example of worldwide presence of *L. monocytogenes* is economical losses that the seafood industry suffers every year in African countries (Ababouch, 2000).

The Ukraine is an example of the countries with a transient economy within the boundaries of the former Soviet Union. Krasovsky et al. (2000) conducted a five-year study on listeriosis in the central and western parts of the Ukraine from 1994 to 1998. Water, soil, clinical samples taken from sick and healthy individuals, tissues of farmed animals, raw food materials, food products, food-graded refrigerators and retail market equipment were the subjects of their extensive research. The results of 8600 environmental samples revealed that *Listeria* species

with both saprophytic and parasitic properties were widely spread in the Ukraine. *Listeria spp.* isolates were found on farms and in meat processing facilities with the incidence being 22.5%. One of the major reservoirs was found to be farm animals infected with listeriosis. The incidences of this infection were registered in more than 80% of sheep and swine production farms. Consequently, researchers found that 7.8% of milk and milk products, 8.8% of meat and fish products, and 6.3% of ready-to-eat and vegetable products were contaminated with *Listeria spp.*. As to the food-graded coolers and retail market equipment, the incidence of contamination reached 2.7%. The infection was mainly (90%) transmitted through consumption of contaminated food products. Among these products were milk products, meat and poultry, vegetables, seafood, eggs, and other food products with the incidences being 41.8%, 32.2%, 10.0%, 8.0%, 2.1%, and 5.9%, respectively. The approximate annual rate of listeriosis was calculated to be within the range of 35 to 70 cases per 100,000 people. Moreover, only 20 % of the infected patients clinically manifested the symptoms.

The virulence of *L. monocytogenes* may vary depending on its strains. Although all thirteen strains of this pathogen are capable of causing listeriosis, the most often infection-associated strains are 1/2 a, 1/2 b, and 4b (McLuchlin, 1990; Farber and Peterkin, 1991). Moreover, the pathogenicity may significantly differ among the isolates themselves. In their studies on virulence of bacterial isolates from the smoked fish industry, Norton et al. (2001) found that some of subtypes of *L. monocytogenes* that persisted in the processing environment possessed attenuated potential pathogenicity which was probably due to some environmental adaptation-associated changes. Bracket and Beuchat (1990) investigated the virulence of *L. monocytogenes* grown on crab meat at 5 ^{o}C and 10 ^{o}C and reported that *L. monocytogenes* maintained its pathogenicity during a two-week period. Their results showed that temperatures

did not affect the pathogenicity and that the virulence was not changed by the length of time (0, 2, 5, 8, 12, and 14 days) the bacterium was grown on either meat samples or tryptose phosphate broth.

Although listeriosis is characterized by low morbidity (approximately 6 cases per one million people), the high percentage of lethality (30%) makes listeriosis one of the most severe food-borne diseases (Rocourt et al., 2003). The incubation period of this foodborne infection that partially contribute to the high mortality may last up to several weeks (Slutsker and Schuchat, 1999).

The ability to survive at a wide temperature range is one of those factors that render *L. monocytogenes* its ubiquitous nature. The optimum temperature for the pathogen is almost within the temperature of a human body (37 °C) (Bell and Kyriakides, 2005). This bacterium is a psychotropic organism that is capable to grow at the refrigeration temperatures (0-8 °C). In the study by Brackett and Beuchat (1990), the increase in population of *L. monocytogenes* inoculated on crab meat by approximately 3 and 5 log was noted at 5 ^{o}C and 10 ^{o}C, respectively during 14-day period. However, the lower refrigeration temperature (~ 4 °C) decreased the growth rate of *L. monocytogenes* to a greater extent than the higher temperature (~ 8 °C) in food products (Guyer and Jemmi, 1991; Rosso et al., 1996). In addition, the other study on naturally contaminated cold-smoked salmon showed that the appropriate refrigeration parameters (<4°C to 5°C) limit the growth of *L. monocytogenes* for approximately a one-month period (Lappi, Ho et al., 2004). Moreover, Guyer and Jemmi (1991) found that freezing contaminated smoked salmon to temperature of – 25 °C did not affect the level of the bacterial pathogen. Mccarthy (1997) also showed that whole boiled crawfish inoculated with 3.0 log of *L. monocytogenes* survived at -20°C. Finally, temperature may affect the ability of this foodborne pathogen to form

biofilms on the two most common surface materials used in food processing plants: stainless steel and polytetrafluoroethylene (PTFE). At lower temperatures (~ 8 °C) *L. monocytogenes* becomes more hydrophilic, which minimizes its ability to colonize the hydrophobic surfaces (PTFE) (Chavant et al., 2002).

The other factor that makes *L. monocytogenes* responsible for a number of preserved product-associated outbreaks is the ability of this pathogen not only to survive but also to grow at elevated concentrations of sodium chloride (~ 10% w/w) (McClure et al., 1989). In addition, the relationship between temperature and salt concentration should be taken into account in terms of detrimental effect on the pathogen, since in contrast to higher temperature the lower one proves to increase the effect of sodium chloride (Nolan et al., 1992). In their studies on the minimal water activity levels for the growth and survival of *L. monocytogenes*, utilizing different humectants (sodium chloride, sucrose, and glycerol), Nolan et al. (1992) found that this bacteria had the minimum water activity level of 0.921, 0.920, and 0.911 in media adjusted with sodium chloride, sucrose, and glycerol, respectively, making *L. monocytogenes* one of the most osmotic-resistant foodborne pathogens.

One of the other growth-limiting factors is pH. Although the optimum value for the growth is neutral or slightly alkaline, *L. monocytogenes* can grow within the pH range of 5.6 – 9.6. Furthermore, some studies have shown that this foodborne pathogen can even resist the pH of less than 5.6 (e.g., coleslaw and cabbage juice) and can be resuscitated from the media, having pH of less than 4.3 (Lou and Yousef, 1999).

L. monocytogenes has been found in various food products, including but not limited to meat and milk products, vegetables, and fruit juices. One of the food categories that have been implicated in listeriosis outbreaks is seafood, particularly, ready-to-eat seafood products (Ryser

and Marth, 1999; Thimothe et al., 2002; WHO/FAO Technical Report, 2004). The prevalence of *L. monocytogenes* in seafood products varies depending on the type of product. For example, cold-smoked fish represents the higher risk than does hot-smoked fish mainly because of specific heat and time treatment parameters applied in the process of production. The cold-smoking process is performed at temperatures ranging from 21.1 to 37.8 °C (70–100 °F) for 12 hours to 5 days, which is a favorable condition for the growth of *L. monocytogenes*; while hot smoking applies 62.8 °C (145 °F) for only 30 minutes (IFT Technical Report 2001). Furthermore, upon their studies on prevalence and growth of this foodborne pathogen in naturally contaminated seafood, Jorgensen and Huss (1998) concluded that such cured seafood as brined shrimps and surimi, caviar as well as marinated herring were of low listeriosis-associated risk compared to the other seafood products, since the growth of this bacterium was mostly inhibited by the low pH (≤5) and some preservatives (e.g., salt, sorbates, or benzoates). When adequate temperature, time, or other preserving parameters are applied during seafood processing, the main cause of contamination is the post-processing handling. For instance, in the experiment on the survival of this pathogen, Mccarthy's results (1997) showed that proper cooking of crawfish inoculated internally or externally with 3.0 log CFU of *L. monocytogenes* reduced the bacterial concentration to nondetectable level, but storing thermally treated crawfish at ≥ 22 °C for a prolonged periods led to an increase of bacterial population. Moreover, the findings of Thimothe et al. (2002) on the detection of *Listeria* in raw, whole, and processed crawfish led to the similar conclusion. Despite of high initial bacterial load of raw crawfish, the heat treatment considerably decreased bacterial contamination to non-detectable levels on the seafood product.

Contamination of finished products occurs during both processing and postprocessing. While the raw material may remain a primary source of *L. monocytogenes* (Eklund et al., 1995),

the processing plant environment might also contribute to the contamination of seafood products (Rorvik et al., 1995; Rorvik et al., 2000; Reij and Aantrekker, 2004). The areas of a processing plant themselves may significantly vary in regard to contamination sources of *L. monocytogenes*. From their studies on contamination pattern of *Listeria* spp. in the smoked salmon processing plant and slaughterhouse, Rorvik et al. (1995) found that the number of *L. monocytogenes*-positive fish samples was highest in the filleting room, whereas no *Listeria* spp. were discovered after-smoking-fish-handling areas of the plant, suggesting that smoking process itself had a deleterious effect on the bacterial quality of the seafood product processed. Moreover, since in contrast to the examined slaughterhouse the smokehouse had the highest level of contamination and slaughtered fish itself was negative for the presence of *L. monocytogenes*, the authors concluded that the smokehouse most probably became a reservoir of this foodborne pathogen (Rorvik et al., 1995). The other studies on the contamination pattern of *L. monocytogenes* in a smoked fish processing environment were conducted by Hoffman et al. (2003). After ribotyping all the isolates of *L. monocytogenes* in order to distinguish between the raw-material associated contamination and environmental one, the authors concluded that the environmental contamination was an independent-type of introduction of *L. monocytogenes* into the seafood products. Furthermore, based on the results of the automated ribotyping applied, it was found that raw fish had greater diversity of strains (16 strains out of 46 isolates) than the processing environment (11 strains out of 115 isolates), indicating separate bacterial populations (Hoffman et al. 2003). Additionally, their results were consistent with findings of other studies, stating that the processing environment possessed specific subtypes of *L. monocytogenes* (Rorvik et al., 2000). As an example of raw-material associated contamination, Thimothe et al. (2002) were able to detect and identify *L. monocytogenes* ribotype DUP-1045B in a drain of the boiling room

of a crawfish processing plant. This ribotype (DUP-1045B) was also found in a smoked fish processing plant (Norton et al., 2001).

The main source of *L. monocytogenes* in the processing environment was found to be drains that accounted for 62.5% of contamination values compared to other environmental sites (e.g., floors, door handles, condensate lines, plastic crates, and equipment) that were responsible for 32.3% of contamination level (Hoffman et at., 2003). The other studies on factors that contribute to contamination of seafood were carried out on forty smoked salmon processing plants (Rorvik et al., 1997). One of the objectives of these studies was the examination of drains for the occurrence of *L. monocytogenes* and other *Listeria species*. *L. monocytogenes* and *Listeria* spp. were detected in the drains of 25 (63%) and 30 (40%) plants, respectively. Rorvik et al. (1997) concluded that the bacterial presence in the drains appeared to be "a sensitive predictor for the presence of *L. monocytogenes* in the smoked salmon". Finally, in his article "Control of *L. monocytogenes* in the food-processing environment" Tompkin (2002) speculates that one of the mechanisms of equipment contamination by floor drains might be through the aerosols that may form during sanitation or air currents.

In contrast to the results of the abovementioned studies, some studies found no *L. monocytogenes* in the drains. Although Peccio et al. (2003) found no presence of the pathogen of concern in the floor drains at meat processing plants; their data might only indicate that good standard sanitation and cleanup procedures were practiced at those plants.

Copper has been utilized for many centuries. But despite of the fact that the antimicrobial effect of this metal was extensively tested on various microorganisms (Gordon et al., 1994 (1,2); Cooney, 1995; Hassen et al., 1998; Cooney and Tang, 1999; Kielemoes and Verstraete, 2001; Beal et al., 2003; Faundez et al., 2004) and that copper has been widely used in

plumbing, medicine, husbandry, and horticulture (Borkow and Gabbay, 2004; Faundez et al., 2004), neither copper nor its alloys have been intensively applied in food industry as a potential means to fight such foodborne pathogen as *L. monocytogenes*. This might be partially due to the fact that scientific data on the effect of copper or its alloys against major pathogens of concern for the food industry, particularly *L. monocytogenes*, are quite limited.

Wilks et al. (2005) compared the antimicrobial properties of different metal alloys that contained various levels of copper to stainless steel and pure copper. They demonstrated that although there was a 5-log reduction in viable cells during the first 48 h on the stainless steel, the population level remained unchanged at 1 x 10^4 during another 26 days. Moreover, they showed that *E. coli* O157:H7 could be recovered in a desiccated state from stainless steel (UNS S30400) after almost one-month period at both refrigerated and room temperatures. The study investigated the bactericidal activity on three brass, five bronze, six copper nickels, and three copper-nickel-zinc alloys. All of the alloys showed a decrease in survival time of *E. coli* O157:H7, and that a higher copper content in the alloy correlated with an increased bactericidal effect. (Wilks et al., 2005). Similar results were found in another study performed by Noyce et al. (2006). In contrast to the artificial enrichment medium, tryptone soya broth, used by Wilks et al. (2005), *E. coli* O157:H7 was grown in extracted beef juice in their experiment. The researchers concluded that viability of this bacterium on copper-containing alloys were dependent on three factors, namely: the composition of alloy, the ambient temperature, and the presence of beef juice. With regards to persistence of viable stains on stainless steel Wilks et al. (2005) found that *E. coli* O157:H7 could survive in a dried state only for the first 6 h at room temperature, regardless of the presence of beef juice. For alloy with the highest content of

copper (95% Cu) 4-log reduction was achieved within 45 min and no viable organism were detected after 75 min without beef juice and 90 min with beef extract (Noyce et al., 2006).

Salmonella enterica and *Campylobacter jejuni* were the subjects of the other experiment that was conducted by Faundez et al. (2004) with the aim of evaluating the antibacterial activity of copper surfaces. The results showed the already-mentioned tendency of copper ions to inhibit the enteropathogens, especially at higher temperature (25 °C). For example, after 8-h exposure to the copper surface at 25 °C, the level of *C. jejuni* reached the ineffective dose of this foodborne pathogen (5-log reduction), whereas at 10 °C only 2-log reduction was noted (Faundez et al., 2004). The study by Abushelabi (2005) on the effect of copper and brass ions on *L. monocytogenes* under different temperatures regimes (4 °C, 25 °C, and 37 °C) showed the equal effect against this foodborne pathogen. Moreover, the researcher identified similar trend as in the experiment by Wilks and Keevil (2004): the higher the temperature the shorter time was needed to reach non-detectable level of *L monocytogenes* both on copper and brass sheets. As an example, 4-log and 6-log reductions of the bacterial population were identified in 4 h at 25 °C and 6 h at 37 °C, respectively; while it took almost 24 h to reach 2- log reduction at 4 °C. These rapid reductions at higher temperature regimes might be due to the fact that the higher the temperatures, the greater amount of copper and brass ions are released from the metal sheets (Abushelaibi, 2005). Additionally, in Abushelabi's studies on the relationship between the antimicrobial copper inhibition and the availability of different nutrients, it was found that the higher concentration of glucose decreased the antibacterial ability of copper ions. Furthermore, replacement of glucose with other sugars (cellobiose, fructose, or mannose) also resulted in the reduced antimicrobial potential of both copper and brass. Also, both high and low levels of various amino acids reduced the inhibitive efficacy of the metal ions. By contrast, the study on

the effect of pH on bactericidal properties of copper or brass revealed no direct relationship between hydrogen ion concentrations and inhibitive properties of the tested metal alloys (Abushelaibi, 2005).

In addition to the effect of copper or copper-containing alloys, the bactericidal effect of copper ions incorporated into paints against various bacteria has been examined by some researchers. Bactericidal activity of copper and non-copper paints was tested by Cooney (1995). A variety of commercial and prototype paints with concentrations of copper being defined as "high" and "low" were tested against both gram-positive (*Staphylococcus aureus* and *Enterococcus faecalis*) and gram negative (*Pseudomonas aeruginosa* and *E. coli*) bacteria. The researcher found that although gram-positive bacteria proved to be more copper-resistant to non-copper latex-formulated paints, almost all the tested copper containing coatings could reduce counts of the bacteria to negligible level in 24 h.

The effect of antifouling marine paints on microbial biofilms developed by *Pseudomonas aeroginosa* on three materials, stainless steel, fiberglass, and aluminum, was investigated by Tang and Cooney (1998). In their study they showed that the maximum population in the biofilms was reached within 1-2 days after inoculation on the coupons that were not painted or coated with marine paint VC-18 without additives. Among the metals tested the biofilms formed on the stainless steel coupons contained higher numbers of total cells and viable cells. Although the paint had no effect on the bacterial population on aluminum and fiberglass, approximately a one-log decrease of viable cells was noted on the steel. Addition of copper to the marine paint at concentration of 19.5% (w/v) resulted in slow biofilm development. Moreover, during the first 24-49 h the coupons coated with copper-containing paint had the number of viable cells that were 1000-fold lower than on the control surface without copper coatings. However, after 72-96

h period the biofilms on copper paint and its control had almost the same number of cells. The authors concluded that biofilm development was inhibited by copper ions only at the beginning; whereas after biofilms reached the equilibrium between viable and total cells, copper could probably be inactivated by either the sequestering process or the growth of copper-resistant cells (Cooney and Tang, 1999).

Copper is known for the production of hydroperoxide radicals that can interact with cell membranes of bacteria and cause cytotoxic effects (Nies, 1999). Furthermore, the copper interaction with nucleic acids and enzyme active sites also lead to bacterial cell death (Cervantes and Gutierrez-Corona, 1994; Lin et al., 1996). To resist the detrimental impact of heavy metals, some protection mechanisms have been adapted by bacteria. Metal exclusion by permeability barriers, active transport of the metal away from the cell, intracellular sequestration of the metal by protein binding, extracellular sequestration, enzymatic detoxification of the metal to a less toxic form, and reduction in metal sensitivity of cellular targets are the major mechanisms contributing to bacterial metal resistance (Hassen et al., 1998; Bruins et al., 2000). *E. coli* was shown to exclude copper ions by alteration of the membrane channel protein porin that led to a decrease in membrane permeability (Bruins et al., 2000). Preventing the interaction of cupric cations with vital cellular components through formation of extracellular polysaccharide coating by *Pseudomonas aeruginosa* is another example of this protection mechanism (Tang and Cooney, 1998). In addition to an ATPase efflux mechanism possessed by *Pseudomonas spp.* (Silver and Phung, 1996); Silver and Ji (1994) found that copper ions are inactivated by periplasmic binding in this bacterium. Intracellular sequestration, a process of metal accumulation within cytoplasm, is a mechanism that protects cellular components from exposure to metal cations. Moreover, in *Pseudomonas spp.* the process of copper sequestration is

achieved by specialized cystein-rich proteins (Silver and Phung, 1996). Synthesis of copper-binding proteins is an example of extracellular sequestration. Unlike *E. coli* and *Bacillus spp.*, some *Vibrio spp.* produce these proteins that detoxify cupric cations in the growth medium (Gordon et al., 1994 (2)).

MATERIAL AND METHODS

Preparation of Coatings with Copper Sulfate

The choice of the paint was based on the type of coating used by the owners of the seafood processing plants involved in the project. Acrylic-epoxy 'Seal-Krete Epoxy Seal' floor paint (Seal-Krete, Auburndale, FL, USA) was used for this research. One container of paint was utilized as a control non-treatment, while another container of paint was mixed by mechanized paint mixer with copper sulfate pentahydrate (Sigma-Aldrich, Inc., St. Louis, MO, USA) that has been shown to exert some antimicrobial activity at very low concentrations, ranging from 5 to 25 ppm against *L. monocytogenes* (Abushelaibi 2005). Although Abushelaibi's experiment demonstrated that even lower concentrations of this copper salt (5–25 ppm) were highly effective against this bacterium, the highest level (25 ppm) of copper sulfate was selected with the aim of reaching the deleterious effects of copper ions within a shorter time period at the sites of seafood processing facilities. In order to reach the level of 25 ppm of this salt (6.36 ppm Cu^{++}) in a one-gallon paint container, 200 ml of coating was replaced with the equal amount of aqueous solution of copper sulfate pentahydrate containing 94.6 mg of this salt. The copper sulfate was well mixed into the floor paint by a shaker for 20 min.

Fabrication of Drain Covers from Copper

Eleven drains covers were fabricated (by Slade Manufacturing, LLC, Baton Rouge, LA, USA) from cold rolled copper plate with a thickness of 3/16 in. (Farmers Copper, Ltd, Galvestone, TX, USA). The plate consisted of 99.9 % copper with hardness and tensile strength being 54 min on the F Rockwell scale and 32,000 psi min, respectively.

Preparation of Concrete with Copper Sulfate

In order to reach 25 ppm of copper sulfate pentahydrate (6.36 ppm Cu^{++}) in the final concrete-water mixture, 96.5 mg of this salt was added to 910 ml of distilled water and, then, the mixture was thoroughly shaken for 20 min. Next, 2,950 g of concrete patch powder (Concrete Patch, Seal Beach, CA, USA) was mixed with 910 ml of water containing the copper salt. The mixture was homogenized with an electric drill for 5 min before the application. Concrete mixed with distilled water only was used as a control.

Seafood Processing Facilities and Preparation of Sampling Sites

Two crawfish and one crab processing facilities located in south Louisiana were collaborated with the study. The plants were designated by letters A, B, or C. The crawfish processing facilities were specialized in the production of cooked peeled tail meat, whereas the crab processing plant processed both crawfish and crab meat. The crawfish growing season of 2006 was extremely dry and the processing was delayed to the beginning of February for all the plants. Full production was commenced in the middle of March, while the peak of the processing occurred through April and May.

Although each plant had its distinctive arrangements and dimensions of processing areas, all three plants had a receiving room, peeling room, packing room, and walk-in cooler. The receiving room was the area where the raw crawfish and crabs were exposed to the thermal treatment (cooking): the crawfish were boiled, while the crabs were steam-cooked under pressure. The peeling and packing rooms were designed for manual peeling and packaging procedures, respectively. Finally, the walk-in-cooler was a blast-type refrigerator in which both raw material and finished seafood products were stored at – 4 ^{o}C.

The sampling sites were prepared during the first week of February 2006 in each processing room. These sites included: one copper drain cover and its control (an original drain cover), a concrete patch with copper ions and its control, a coating with copper ions and its control. At each plant the copper-made drain covers were thoroughly cleaned with 70 % ethanol immediately before placing them. The control drain covers differed from each other in materials they had been made from within each plant. Some covers were fabricated from steel, whereas the others – from plastic. Four copper drain covers were used in each plant, except for the crab processing plant, which had only three copper drains since there was no drain in the walk-in cooler of this facility.

All of the copper-containing and control concrete patches (20 x 20 cm) and copper-containing and control coatings (30 x 30 cm) were placed on the floor in such a way that production water would not transfer copper ions from the copper-containing sites to control sites. The control and copper-containing sites were either placed within a reasonable distance from each other or the control sampling sites were situated at the higher floor level than the copper-containing sites, thus preventing a possible copper ion contamination.

The sampling sites in the receiving rooms of two crawfish processing plants were located under the front part of boiling equipment in order to better evaluate the effectiveness of copper ions against the bacteria introduced with raw material. In the receiving room of the crab processing facility sampling sites of coatings and concrete were placed within the area where the raw material was transferred from the containers into the steamers, thus allowing to better evaluate the antimicrobial effectiveness of coatings and concrete containing copper ions. The control and copper-containing

sampling sites in the peeling rooms of three facilities were located under two separate tables, on which manual peeling was regularly performed. In the packing rooms of plants B and C all the sampling sites were placed under the tables on which the weighing and packing occurred, while at plant A the control and copper-containing coatings were laid under the cooling tank with the other sampling sites being put under the tables. In the walk-in cooler of plant A the concrete patches were placed on the floor under the shelves, while the coatings were laid in the open area by a wall. In the refrigerators of plants B and C all the sampling sites were placed on the floor by walls. Thus, each processing facility had four copper-containing coating and four copper-containing concrete sampling sites. Some sample drains, coatings, and concrete patches together with their control counterparts can be seen in the pictures provided in Appendix 1.

Sampling Procedure

For surface bacteriological assessment the swab-rinse method was applied by means of cotton-tipped swabs and two media (Favero et al., 1984). Brain heart infusion (BHI, Difco, MD, USA) broth was used for collection of *Pseudomonas spp., E.coli* and other coliforms as well as for determination of total aerobic count, whereas University of Vermont modified Listeria enrichment medium (UVM, Difco, MD, USA) was utilized for isolation of *Listeria spp*..

At each plant, swabbing was performed before the processing of seafood every other week in the course of two months. Templates with openings of 16 square cm that corresponded to the area to be swabbed at each sampling site were prepared. Before each swabbing, the templates were sterilized with 70% alcohol. While swabbing the swab plastic shafts were held at approximately 30 degree angle contact with the examined

surface. Then, the exposed swabs were returned to the tubes with the broths. Finally, the centrifuge tubes with samples were placed in an insulated foam box with ice packs and delivered to the walk-in cooler at LSU Ag Center Food Microbiology and Safety Laboratory (Baton Rouge, LA, USA). All the samples were analyzed within 24 h.

Plating Procedure

In order to dislodge collected microorganisms from the cotton fibers each tube was vigorously vortexed for 15-20 s immediately before plating. To determine the presence of *Pseudomonas aeroginosa* in the environmental samples, the ten-fold serial dilutions were prepared in phosphate buffer saline diluent (PBS). Next, 0.1 ml of aliquot from each dilution was spread plated onto 'Pseudomonas Agar F' medium (Flo Agar, Difco, MD, USA) in duplicates. The plates were incubated at 35±2 ^{o}C for 18-24 h. If the isolates failed to grow or grew slowly, the plates were reincubated at 25 ^{o}C for 48 h. After the incubation period the colonies grown on the plates were examined under the wavelength of 254 nm of UV light for fluorescin, a greenish-yellow fluorescent pigment, and the fluorescing colonies were counted. The counts were averaged and reported as log CFU/cm^2.

Total aerobic microbial counts were determined on plate count agar (Difco, MD, USA). The serial dilutions were shaken on the vortex for 7 s before plating. The plates were incubated 35±2^{o}C for 48 h and averaged counts were recorded as log CFU/cm^2.

'3M Petrifilm *E. coli*-Coliform Count Plates' (3M, MN, USA) were used to determine *E. coli* and coliforms. The decimal dilutions were prepared with PBS diluent and 1 ml of each dilution was pipetted onto the center of appropriately labeled petrifilm plate. The top film of the plate was released and the spreader with the ridge being side

down was gently pressed on the top film inoculum. After the gel of the system became solidified the plates were incubated at 35±2 ^{o}C for 48 h. Red colonies with air bubbles of *E.coli* and red colonies of coliform were counted and recorded as log CFU/cm^2. The plating was performed in duplicates.

To detect *Listeria spp.* from the environmental samples 'Oxford Listeria Agar Base' medium (Acumedia, MI, USA) and selective supplement, 'Listeria Supplement SR0140E' (Oxoid, England, UK) were utilized. From the UVM broth the serial dilutions were prepared and 0.1 ml of aliquot from each decimal dilution was plated onto the duplicate plate of oxford medium. After spreading the inoculum the plates were inverted and incubated at 35±2 ^{o}C for 48 h. Black colonies were counted on the Quebec counter and the results were expressed as log CFU/cm^2.

Confirmation of *L. monocytogenes* by PCR Analysis

The UVM broth samples that produced black colonies morphologically representing *Listeria spp.* on Oxford plates were incubated at 35 ± 2 ^{o}C for 36-48 h. Five hundred µl of each presumably *L. monocytogenes*-positive enriched broth were then lysed in the waterbath at 94 ± 1 ^{o}C for 5 min. The cell lysate of strain 1/2a of *L. monocytogenes* V 7 (CDC, Atlanta, GA, USA) was run as a control.

The primers MLis-1 and MLis-2 with the sequences and locations being 5'-GATGCATCTGCATTCAATAAAGAAA-3' (126-151) and 5'-TGTCACTGCATCTCCGTGGTATACTA-3' (309-284) (synthesized by Integrated DNA Technologies, Coralville, IA, USA) targeting listeriolysin O (*hlyA*) gene sequence were used for this research. The PCR reaction was performed at 95 ^{o}C for 30 s, 60 ^{o}C for 30 s, and 72 ^{o}C for 30 s with the total number of cycles being 35 (Hsih and Tsen, 2001).

The first denaturation and last extension steps were performed for 10 min. Next, the amplified PCR products with molecular weight of 184 bp were examined through electrophoresis with 2 % agarose gel using Tris-acetic acid-EDTA buffer at 80 V for 2 h. The resultant bands were stained with SYBR Green I nucleic acid gel stain and, then, visualized by UV transillumination.

Statistical Analysis

The data were subjected to JMP statistical software (Version 4.0.3, SAS Institute Inc., Cary, NC, USA) for analysis of variance (ANOVA). The Student's *t* test was applied for pairwise comparisons. Significant differences are presented at a 95 % confidence level ($P \leq 0.05$).

RESULTS AND DISCUSSION

The ability of some foodborne pathogens to survive for extended time periods on the surfaces of many materials used in seafood industry has been reported (Wong, 1998; Bagge-Ravn et al., 2003; Kusumaningrum et al., 2003). *L. monocytogenes* has been found to bind either reversibly or irreversibly to a variety of materials representing metals, rubbers, and polymers with different adhesive strength (Beresford et al., 2001). Once attached to a surface the extracellular material of *L. monocytogenes*, listerial adhesin mediates biofilm formation. Compared to planktonik bacteria, *Listeria* species in biofilms are more resistant to sanitizers and heat (Taormina and Beuchat, 2002). Moreover, the bacterial resistance to adverse environments depends on such factors as temperature, relative humidity, surface type, and soil presence. The highest incidence of microbial biofilms is found on wet locations such as wet floors, especially floor cracks, and drains of processing facilities (Wong, 1998; Chavant et al., 2002). Although there is no consistent relationship between disease incidence and enhanced biofilm formation, the environmental contamination remains one of the factors contributing to contaminating product contact surfaces and finished food products (Borucki et al., 2003; Chen et al., 2003; Hoffman et al., 2003). Thus, one of the possible approaches to diminishing the risk of this environmental contamination pattern is to reduce both transient and highly persistent residual foodborne pathogens in drains and floor cracks. For this purpose copper metal and copper sulfate pentahydrate were applied in this research.

The ions of heavy metals have been known for their bactericidal and bacteriostatic effects and, therefore, are applied in various fields including medicine, plumbing, animal husbandry, horticulture, and others (Lin et al., 1996; Beal et al., 2003; Borkow and Gabbay, 2004). In addition to the interactions of cupric ions with nucleic acids and enzyme active sites, their

positively charged ions attach onto the negatively charged bacterial cell walls, thus destroying its permeability and that leads to cell death and lysis (Cervantes and Gutierrez-Corona, 1994; Lin et al., 1996).

Table 1.1. Antimicrobial effect of copper-fabricated drains against *Listeria spp.*, *Pseudomonas spp.*, total aerobic bacteria, and coliforms.

Plants	Bacterial mean counts, log CFU/cm^2							
	Listeria spp.		*Pseudomonas spp.*		Total aerobic count		Coliforms	
	Copper drain	Control	Copper drain	Control	Copper drain	Control	Copper drain	Control
A	0.80±1.01	2.02±1.05	1.06±1.41	1.52±1.76	3.35±1.58	4.22±1.71	-	-
B	1.34±0.97	2.47±1.24	1.91±1.68	2.10±1.70	3.88±1.81	4.70±2.09	-	-
C	1.63±0.92	2.75±0.73	1.89±1.47	2.67±1.66	3.19±1.44	5.12±0.78	-	-
All plants	1.22±1.01	2.38±1.07	1.59±1.55	2.04±1.73	3.50±1.62	4.64±1.68	0.48±0.68	0.56±0.71

The antimicrobial effect of copper-made drains was investigated in the course of six-week seafood processing period at two crawfish and one crab processing plants. The data provided in Table 1.1 represent the bacterial counts of *Listeria spp.* determined on the copper-fabricated drain covers and their controls. The mean count of *Listeria spp.* (0.80 log CFU/cm^2) found on the copper drain covers of all processing rooms at plant A was statistically significantly different from that of the control drains (2.02 log CFU/cm^2) with the reduction of 1.22 log CFU/cm^2. The same anti-*Listeria* effect of copper drains can be seen in the other two processing environments. On average, 1.12-log CFU/cm^2 bacterial reduction was observed at both plant B and C. Although *Listeria spp.* were found on all copper drains, the overall reduction found in all three seafood facilities might have indicated that copper drains were quite effective against *Listeria* family.

During the processing of seafood various and numerous bacteria are persistently introduced into and transferred from one location to another with raw materials and by employee

traffic within processing areas. In our research, at each plant the sampling took place before the actual processing was started and, therefore, there was approximately a 10 h non-processing period (the time from when the processing stops till it resumes next morning) during which bacteria were not presumably introduced onto the drains or floors. In other words, it probably took daily about 10 h for the copper ions of the drain grills to diminish the load of *Listeria spp.* introduced during seafood production by about one log reduction at all the facilities. This reduction was much lower than that obtained under laboratory conditions. Previous research on the bactericidal effect of copper metal on *L. monocytogenes* revealed a 4-log reduction within 4 h and showed that 8 h was enough to reach non-detectable level of the bacterium exposed to copper metal sheets at ambient temperature (Abushelabi, 2005). This discrepancy in microbial reductions observed at the plants and laboratory might be partially explained by the higher copper resistance of *Listeria* biofilms compared to planktonik cells of *L. monocytogenes*. At the processing plants the bacteria were able to form these biofilms on the surfaces of the sampling sites during at least one-month period (the drains were placed about one month prior to sampling procedures). On the other hand, the lab study did not allow enough time for planktonik cells of *L. monocytogenes* to protect themselves by developing biofilms. According to Kielemoes and Verstraete (2001), copper is shown to impede biofilm formation only during the first 48 h, while after this period the copper effect on the microbial adhesion disappears.

Table 1.1 also shows the counts of *Pseudomonas spp.* on the copper and control drains. There was no significant difference detected between the mean of bacterial counts found on all copper covers and that of their controls in any of the seafood plants, suggesting that drains covers seemingly had no detrimental effect on this spoilage bacterium. This partially can be explained by copper resistance that bacteria are able to acquire within a short period of time. In

their study on microbial copper-resistance, Tang and Cooney (1998) showed that after the application of tryptic soy agar that contained 20 mM copper (II) and killed about 90% of *Pseudomonas aeruginosa*, almost 100% of the viable cells taken from developed biofilms at 6 h possessed copper resistance. These resistant cells can possibly form an outer layer that would protect copper-sensitive cells that grow inside a biofilm (Tang and Cooney, 1998).

Regarding the effect of copper metal on the total aerobic count there was approximately a 2 log CFU/cm^2 decrease in the bacterial population at plant C (Table 1.1). Despite of the fact that there were no significant differences between the mean of total aerobic counts on copper drains and that of their control counterparts at either plant A or B, the combined data of the three processing facilities showed that the copper drains reduced the overall total aerobic load by over 1 log CFU/cm^2 (Table 1.1).

Table 1.2. Antimicrobial effect of copper coatings against *Listeria spp.*, *Pseudomonas spp.*, total aerobic bacteria, and coliforms.

Plants	Bacterial mean counts, log CFU/cm^2							
	Listeria spp.		*Pseudomonas spp.*		Total aerobic count		Coliforms	
	Copper paint	Control	Copper paint	Control	Copper paint	Control	Copper paint	Control
A	1.50±1.27	1.44±1.13	0.81±1.52	1.57±1.61	3.55±1.69	3.68±1.53	-	-
B	1.95±1.55	2.23±1.27	1.35±1.06	1.96±1.53	3.93±1.65	4.16±1.84	-	-
C	1.99±1.29	2.12±1.60	1.56±1.70	2.12±1.83	3.97±1.01	4.84±1.43	-	-
All plants	1.80±1.37	1.92±1.35	1.23±1.44	1.88±1.63	3.81±1.48	4.20±1.65	0.44±0.56	0.50±0.73

Since all the processing plants appeared to be clean in terms of the presence of *E. coli* at all sampling sites including drains, there was not enough evidence on any effect of copper ions against this bacterium at these processing facilities (Appendix 2, Table 2.4). Nevertheless, based on the data obtained in some previous studies, the copper-fabricated drains could be used in the food processing environment because of bactericidal properties against this bacterium, especially

E. coli O157:H7. Wilks and Keevil (2004) conducted their experiment on the effect of copper alloy surfaces on the viability of *E. coli* O157:H7. They tested 25 different copper-, brass-, bronze-, copper-nickel-, and copper-nickel-zinc-based alloys as well as stainless steel. Although the alloys exhibited different antimicrobial effects, a few general trends were pointed out. The inhibition effects of all metal alloys increased as either the temperature (from 4°C to 20°C) or copper concentration increased. The brass-based alloys mostly exhibited less antimicrobial effect than that of copper alloys. It took 1-6 h and minimum 3 h for all types of copper alloy surfaces to reduce the pathogen to non-detectable level at 20°C and 4°C, respectively (Wilks and Keevil, 2003, 2004).

The data obtained from each processing environment are also not sufficient to determine the efficacy of copper ions at all sampling sites against coliforms (Tables 1.1, 1.2, 1.4). However, based on the combined data from three processing plants no significant difference was determined between the overall mean of coliform counts on the copper drains located at all the plants and that of their corresponding controls.

In addition to the bactericidal effect of copper metal, the antimicrobial properties of copper ions incorporated into coatings have been examined. All the copper-containing coatings proved to be ineffective against *Listeria spp.*, *Pseudomonas spp.*, coliforms, and other aerobic bacteria (Table 1.2). The previous study (Abushelaibi 2005) conducted under laboratory conditions indicated that copper ions could be quite effective against *L. monocytogenes* at very low levels of copper sulfate pentahydrate ranging from 5 to 25 ppm in coatings. However, in this study the chosen concentration of 25 ppm of this salt (6.36 ppm Cu^{++}) was not enough for copper ions to exert antimicrobial properties in the seafood processing environment (Table 1.2). The insufficient amount of copper ions might not be the single reason of copper-containing paint

inefficacy. Although Tang and Cooney (1998) examined the antimicrobial effect of paint that had much higher level of copper (19.5% (w/v)) against *Pseudomonas aeruginosa* the obtained results were quite similar to ours. After 5-6 days viable cell counts on the copper marine paint and its control were equivalent. In addition to copper resistance that the bacterium could have acquired, the researchers suggested that copper might have been inactivated by bacterial sequestration of copper ions (Gordon et al., 1994 (2); Silver and Phung, 1996).

Table 1.3. Antimicrobial effect of copper concrete against *Listeria spp.* and *Pseudomonas spp.*.

Plants	Bacterial mean counts, log CFU/cm^2					
	Listeria spp.			*Pseudomonas spp.*		
	Copper concrete	Control concrete	Original floor	Copper concrete	Control concrete	Original floor
A	1.07±0.93	0.76±0.97	1.25±1.13	0.76±1.17	0.90±1.20	1.34±1.77
B	1.53±1.49	1.85±1.45	3.10±1.32	0.92±1.12	1.42±1.47	1.82±1.67
C	1.71±1.52	1.97±1.81	2.00±1.66	1.29±1.56	1.09±1.54	1.23±1.67
All plants	1.43±1.34	1.53±1.53	2.19±1.56	0.99±1.29	1.38±1.40	1.46±1.69

Table 1.4. Antimicrobial effect of copper concrete against total aerobic bacteria and coliforms.

Plants	Bacterial mean counts, log CFU/cm^2					
	Total aerobic counts			Coliforms		
	Copper concrete	Control concrete	Original floor	Copper concrete	Control concrete	Original floor
A	3.05±1.70	3.26±1.26	3.77±1.35	-	-	-
B	3.74±1.97	4.08±2.18	4.67±1.85	-	-	-
C	4.43±1.39	4.42±1.72	5.19±1.16	-	-	-
All plants	3.74±1.76	3.92±1.79	4.52±1.56	0.34±0.52	0.43±0.66	0.34±0.60

Another objective of this research was to investigate the bactericidal or bacteriostatic effect of concrete patches containing copper ions in the form of copper sulfate pentahydrate. The

concentration of 25 ppm of the salt (36 ppm Cu^{++}) was tested against two controls, concrete containing no copper salt (control A) and original concrete floor of the plants (control B). Almost all sampling sites of copper-containing concrete showed no antibacterial effect in the seafood processing facilities compared to control A (Tables 1.3 and 1.4). The exceptions included the copper patches and non-copper patches (controls A) that showed 0.71 log CFU/cm^2 reduction of *Listeria spp.* compared to controls B at all the plants. In addition, the copper and non-copper concrete patches reduced the total aerobic bacteria counts by 0.69 CFU/cm^2 log in three processing facilities compared to the original floor controls (Table 1.4). These reductions caused by the concrete with or without copper ions clearly indicated that not the copper ions but rather the relatively freshly laid concrete itself proved to be toxic to some extent towards the bacteria tested. The alkaline pH of concrete might have been a major contribution to the antimicrobial toxicity exerted by the copper and non-copper concrete patches at the plants (Oregon Department of Transportation, 2003).

All the PCR samples were negative for detection of *L. monocytogenes* in UVM broths except for the sample taken from the copper-fabricated drain situated in the peeling room at plant B, meaning that the black colonies grown on Oxford plates represented *Listeria species* other than *L. monocytogenes* (Table 1.5). In addition, these negative PCR results suggested that the effective clean-up and standard sanitation procedures were practiced at all plants involved in the project.

The relatively unequal conditions of some sampling sites and their controls due to the specificity of processing plant arrangements constitutes the major drawback of this experiment. For example, the drain with copper grill that was situated closer to the boiling equipment than its control in the receiving room at plant B drained much more liquid from raw seafood material

immersed into processing water, suggesting the higher contamination by the pathogens (See picture 1 of Appendix 1). On the other hand, the main advantage of this research is that it has been performed not under the strict laboratory conditions but rather actual seafood processing conditions encountered daily in the crawfish and crab production environments. This has enabled us to better evaluate the actual bactericidal effect of copper-fabricated drains, coatings and concrete containing copper ions in combating some of the dangerous foodborne pathogens that pose both health and economic threat to the consumers and seafood producers, respectively (CDC, 2001). Despite of the fact that neither coatings nor concrete that contained copper sulfate pentahydrate proved effective against the tested bacteria, the possibility of their application as one of approaches to controlling environmental contamination should not be disregarded. Future work should be done in this field by determining the minimal level of copper sulfate pentahydrate in the paint and concrete that will be able to effectively reduce bacterial contamination. Moreover, future studies should not exclude the possibility of other bactericidal and bacteriostatic forms of copper that can be incorporated into coatings and concrete. This may include the application of other copper salts or copper shavings. Subsequently, the effective minimal inhibitory concentrations of the copper forms should be determined both at the laboratory and seafood processing plants.

Table 1.5. Results of PCR analysis on the presence of *L. monocytogenes* in UVM samples taken at the processing plants. [a]

Processing area	Sampling sites	PCR results											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper drain covers	-[b]	-	-	----	-	-	----	-	-	----	-	-
	Control drain covers	-	-	-	-	-	-	-	-	-	----	-	-
	Copper-containing coating	-	-	-	-	-	-	-	-	-	----	-	----
	Control coating	-	-	-	-	-	-	-	-	-	----	-	----
	Copper containing concrete	-	-	-	-	-	-	-	-	-	----	-	----
	Control concrete	----[c]	-	-	-	-	-	-	----	-	----	-	----
	Original floor	-	-	-	-	-	-	-	-	-	-	-	-
Peeling room	Copper drain covers	-	-	-	----	+	-	----	----	-	----	----	----
	Control drain covers	-	-	-	-	-	-	-	-	-	----	-	-
	Copper-containing coating	-	-	-	-	-	-	-	----	-	-	----	----
	Control coating	-	-	-	-	-	-	-	-	-	-	-	----
	Copper containing concrete	-	-	-	-	-	-	-	-	-	-	-	-
	Control concrete	-	-	-	----	-	-	-	-	-	-	-	-
	Original floor	-	-	-	----	-	-	-	-	-	-	-	-
Packaging room	Copper drain covers	-	-	-	----	----	-	-	----	-	----	----	-
	Control drain covers	-	-	-	-	-	-	-	----	-	-	-	-
	Copper-containing coating	----	-	-	----	----	-	-	----	-	----	-	-
	Control coating	----	-	-	----	-	-	-	----	----	-	-	-
	Copper containing concrete	----	-	-	----	----	-	-	----	----	----	----	-
	Control concrete	----	-	-	----	----	-	-	----	----	----	----	-
	Original floor	----	-	----	----	-	-	-	----	-	----	-	-
Walk-in cooler	Copper drain covers	-	-	----	-	-	----	----	-	----	-	-	----
	Control drain covers	-	-	----	-	-	----	-	-	----	-	-	----
	Copper-containing coating	-	-	----	-	----	-	----	-	----	-	-	-
	Control coating	-	-	----	-	-	-	-	-	----	-	-	-
	Copper containing concrete	----	-	-	-	----	-	-	----	----	-	-	-
	Control concrete	----	-	----	----	-	-	----	-	----	-	-	-
	Original floor	-	-	----	-	-	-	----	-	----	-	-	----

[a] Only presumably *L. monocytogenes*-positive UVM enriched broths (the broths that yielded black colonies on Oxford medium) were PCR tested.
[b] Symbols "-" and "+" indicate that PCR results were either negative or positive, respectively.
[c] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

CONCLUSION

The antibacterial properties of copper-fabricated drains, coatings and concrete containing cupric ions in the form of copper sulfate pentahydrate have been evaluated against *Listeria spp.*, *Pseudomonas spp*., *E. coli* and other coliforms, and total aerobic bacteria in one crab and two crawfish processing facilities over two months. The findings revealed that no bactericidal effect of copper drains against *Pseudomonas spp.* and coliforms was observed. However, the copper drain grills maintained a 1.16 log and 1.14 log CFU/cm^2 decrease in *Listeria* population and total aerobic count, respectively, when compared to non-copper control drains.

Regarding the antibacterial activity of copper drains, coatings and concrete against *E. coli*, there was not enough supportive evidence as to any interaction between copper ions and this organism. This was most probably due to good personal hygiene practiced by employees of the seafood processing plants and good standard sanitation and clean-up procedures performed in these facilities

In contrast to some previous studies on copper paint (Abushelaibi 2005), this research found that coatings containing 25 ppm of copper sulfate pentahydrate proved to be ineffective against all the bacteria tested. The failure of copper paint to exert antimicrobial activity might be partially explained by the low level of copper ions in the coatings (6.36 ppm Cu^{++}). Additionally, the inactivation of copper ions by bacterial sequestration process and the ability of bacterial cells to become copper-resistant within a short period of time might also have made their contribution.

Finally, the antimicrobial effect of concrete containing copper ions was investigated in the seafood processing environment. The data demonstrated the antimicrobial inefficacy of copper concrete compared to freshly placed non-copper concrete sites. However, when

compared to the counts found on the original floor controls both concrete patches with and without cupric ions showed some bactericidal or bacteriostatic effect by maintaining 0.71 log and 0.69 log CFU/cm^2 reductions in *Listeria* population and total aerobic bacteria, respectively. This antimicrobial activity should not be attributed to copper ions. The slightly alkaline pH of relatively freshly laid cement might have been partially accountable for these bacterial reductions.

Based on the overall results, the copper-fabricated drains could be used in the seafood processing facilities as one of the approaches for reducing environmental contamination by spoilage and pathogenic bacteria. Regarding the application of copper coatings and concrete at the seafood production plants, future research should be conducted to find the most effective bactericidal copper forms and their effective minimal inhibitory concentrations.

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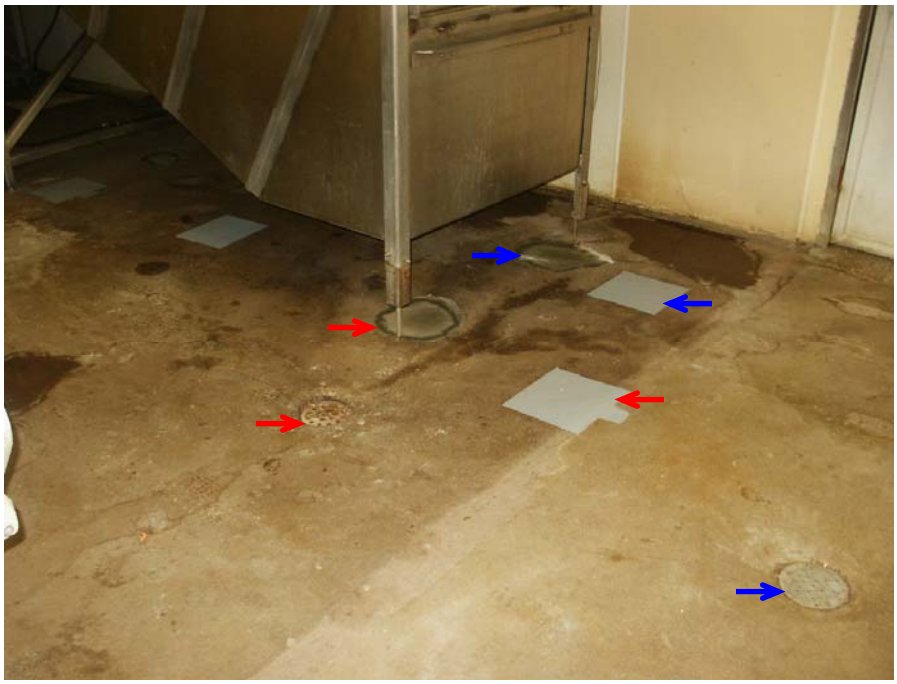
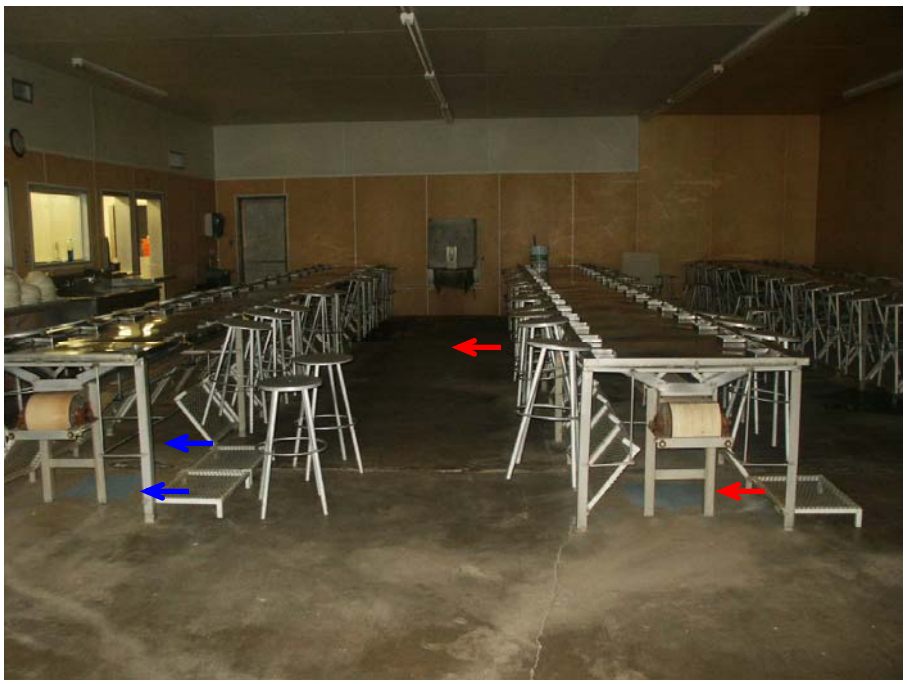
APPENDIX 1

SAMPLING SITES IN THE PROCESSING ROOMS OF SEAFOOD FACILITY B

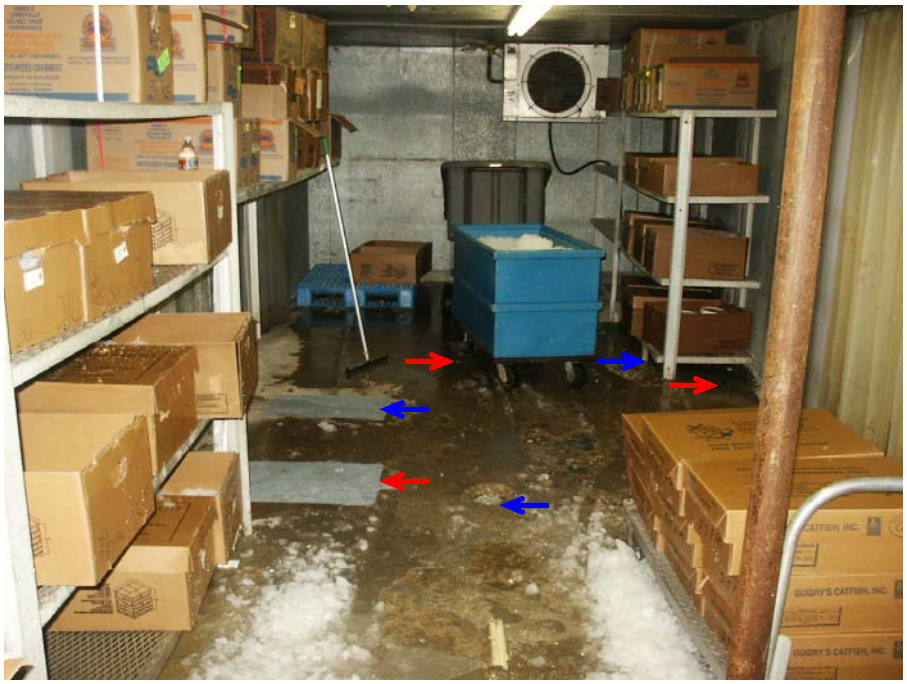


Picture 1. The sampling sites located by the boiling equipment in the receiving room of plant B. Red arrows indicate copper-containing sites and blue arrows – their controls.

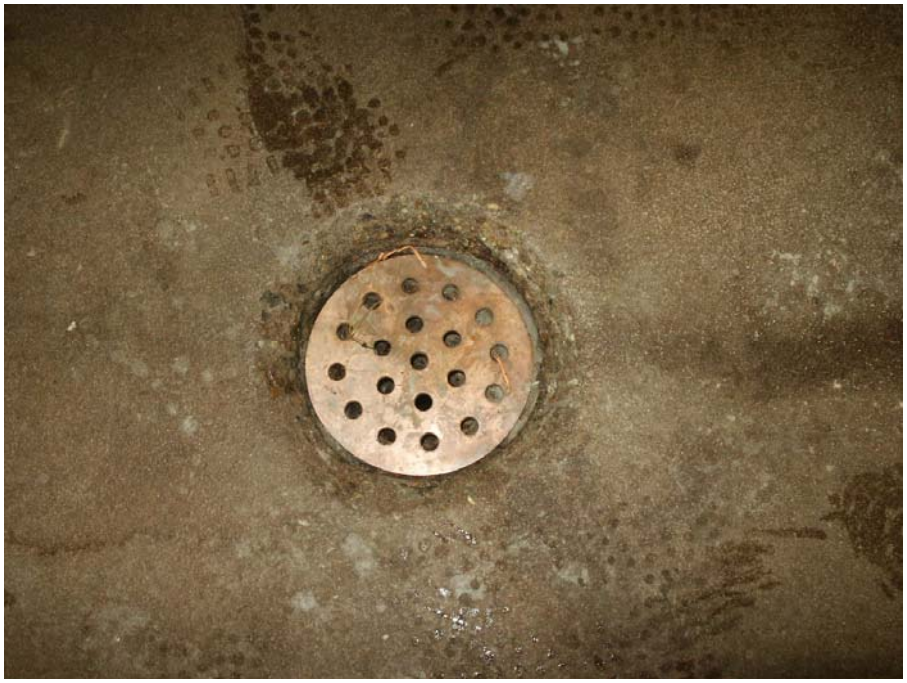


Picture 2. The sampling sites located under the peeling tables in the peeling room of plant B. Red arrow indicates a copper-containing site and blue arrows – controls.

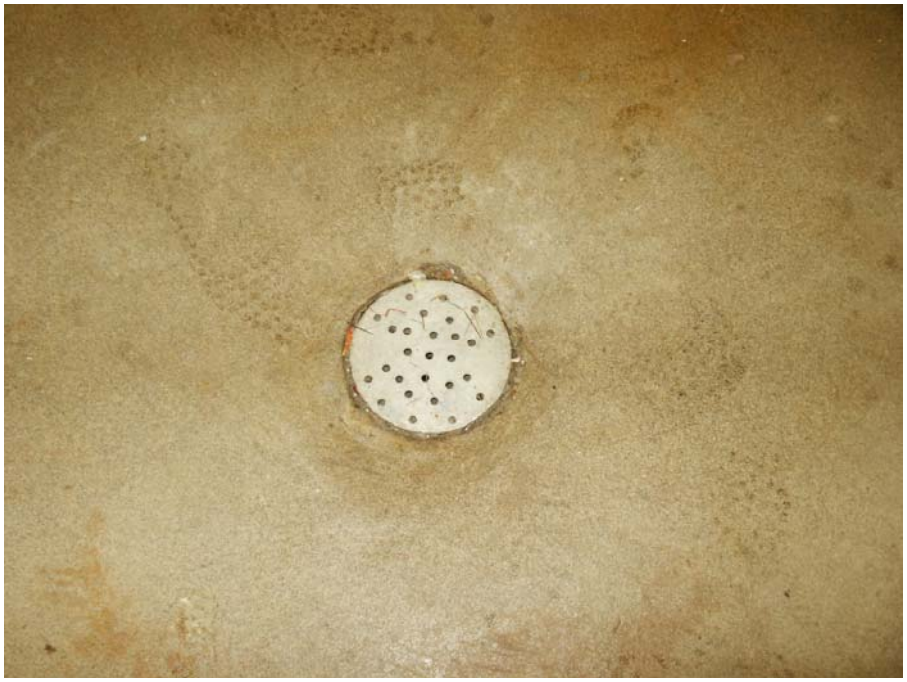
Picture 3. The sampling sites located under the weighing table in the packaging room of plant B. Red arrows indicate copper-containing sites and blue arrows – their controls.



Picture 4. The sampling sites located by the walls and under the shelves in the walk-in cooler of plant B. Red arrows indicate copper-containing sites and blue arrows – their controls.



Picture 5. A copper-fabricated drain cover.



Picture 6. An original aluminum drain cover that served as a control.

APPENDIX 2

TABLES REPRESENTING COUNTS OF THE TESTED BACTERIA AT THE SAMPLING SITES

Table 2.1. Bacterial counts of *Listeria species* on copper drain covers and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper drain covers	1.57	1.36	2.27	ND[a]	2.73	2.16	ND	1.39	3.19	ND	2.80	1.74
	Control drain covers	2.16	2.36	2.54	3.94	2.33	3.72	2.05	1.98	3.31	ND	3.97	2.60
Peeling room	Copper drain covers	2.06	1.66	2.20	ND	2.23	2.09	ND	0.97	ND	ND	ND	ND
	Control drain covers	1.80	2.73	2.45	1.27	4.48	3.19	1.64	1.77	2.97	ND	4.05	4.09
Packaging room	Copper drain covers	1.03	1.14	1.39	ND	ND	1.49	2.53	ND	1.19	ND	ND	1.79
	Control drain covers	2.13	1.89	2.36	1.77	1.57	1.67	3.52	ND	2.27	2.39	0.49	1.79
Walk-in cooler	Copper drain covers	1.57	1.36	----[b]	1.33	1.69	----	ND	2.60	----	2.66	1.49	----
	Control drain covers	2.44	2.60	----	1.97	3.86	----	2.09	2.97	----	3.19	2.49	----

[a] ND – non-detectable bacterial level in a sample.
[b] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

Table 2.2. Bacterial counts of *Pseudomonas species* on copper drain covers and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper drain covers	0.49	3.27	2.97	0.49	3.19	0.79	ND	4.19	3.79	ND	ND	ND
	Control drain covers	ND[a]	4.19	3.33	ND	3.39	ND	ND	1.19	4.09	1.49	ND	ND
Peeling room	Copper drain covers	1.19	1.97	1.49	1.49	2.49	ND	ND	0.49	3.97	ND	ND	1.39
	Control drain covers	1.97	1.79	2.97	ND	4.90	3.79	1.09	2.79	3.44	ND	ND	ND
Packaging room	Copper drain covers	1.64	1.89	0.49	ND	1.53	1.53	1.44	0.49	2.49	ND	ND	3.79
	Control drain covers	4.34	2.67	3.72	ND	2.79	3.49	1.49	ND	2.79	3.76	ND	4.39
Walk-in cooler	Copper drain covers	4.49	4.72	----[b]	1.79	1.79	----	ND	4.49	----	3.97	ND	----
	Control drain covers	4.87	3.87	----	1.49	3.49	----	ND	2.49	----	3.89	ND	----

[a] ND – non-detectable bacterial level in a sample.
[b] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

Table 2.3. Total aerobic counts on copper drain covers and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper drain covers	3.92	4.56	2.97	3.27	7.66	4.27	1.33	4.63	4.15	4.19	5.69	3.97
	Control drain covers	4.46	5.66	4.21	6.17	7.99	5.33	1.44	4.50	5.19	4.49	6.44	5.81
Peeling room	Copper drain covers	6.47	4.16	3.53	4.10	5.27	0.49	2.97	0.49	4.21	0.49	2.79	2.19
	Control drain covers	5.48	4.58	4.89	3.19	6.25	5.33	5.68	0.79	5.41	ND[a]	5.95	6.94
Packaging room	Copper drain covers	3.19	3.14	0.49	3.49	1.44	3.09	3.27	1.49	4.39	0.49	3.64	4.57
	Control drain covers	4.41	3.61	5.02	5.90	3.07	3.97	4.77	0.97	4.39	2.19	2.53	4.92
Walk-in cooler	Copper drain covers	3.19	5.17	----[b]	3.49	4.59	----	3.27	2.81	----	0.49	4.57	----
	Control drain covers	4.41	7.55	----	5.90	5.03	----	4.77	4.96	----	2.19	5.48	----

[a] ND – non-detectable bacterial level in a sample.
[b] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

Table 2.4. Bacterial counts of *E. coli* on all sampling sites at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper drain covers	ND[a]	ND	ND	ND	0.49	ND	ND	ND	ND	ND	ND	ND
	Control drain covers	ND	1.27	ND	ND	0.49	ND	ND	ND	ND	1.09	ND	ND
	Copper-containing coating	ND	1.19	ND	ND	ND	ND	ND	ND	ND	ND	1.67	----
	Control coating	ND	0.49	ND	3.99	1.49	ND	ND	ND	1.09	ND	2.12	----
	Copper containing concrete	ND	0.49	ND	ND	ND	ND	ND	ND	ND	ND	2.10	ND
	Control concrete	0.49	ND	ND	ND	ND	ND	ND	ND	0.49	ND	0.49	ND
	Original floor	ND	0.49	ND	ND	ND	ND	ND	0.79	ND	ND	1.09	ND
Peeling room	Copper drain covers	ND	1.09	ND	ND	0.49	ND	ND	ND	ND	ND	ND	ND
	Control drain covers	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Copper-containing coating	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	----
	Control coating	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	----
	Copper containing concrete	ND	0.49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control concrete	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.49	ND
	Original floor	ND	1.19	ND	ND	ND	ND	ND	ND	ND	ND	0.49	ND
Packaging room	Copper drain covers	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control drain covers	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Copper-containing coating	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control coating	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Copper containing concrete	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control concrete	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Original floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Walk-in cooler	Copper drain covers	ND	ND	----[b]	ND	ND	----	ND	0.79	----	0.49	ND	----
	Control drain covers	ND	ND	----	ND	0.79	----	ND	ND	----	ND	0.99	----
	Copper-containing coating	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control coating	ND	0.49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Copper containing concrete	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control concrete	ND	3.99	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Original floor	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

[a] ND – non-detectable bacterial level in a sample.
[b] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

Table 2.5. Coliform counts on all sampling sites at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper drain covers	1.09	1.67	ND	0.97	2.53	ND	ND	0.49	ND	ND	ND	ND
	Control drain covers	1.33	0.79	0.79	ND	2.19	ND	ND	0.79	ND	ND	ND	0.79
	Copper-containing coating	1.27	0.97	0.79	ND	2.09	ND	ND	0.79	0.49	0.79	1.19	----
	Control coating	1.39	1.19	0.49	ND	2.49	ND	ND	ND	0.49	ND	0.49	----
	Copper containing concrete	1.60	1.33	0.79	0.49	1.79	ND	ND	ND	0.49	ND	0.79	ND
	Control concrete	1.33	1.44	0.49	0.49	1.97	ND	ND	0.79	ND	ND	1.57	1.09
	Original floor	0.79	1.27	0.49	0.49	2.19	ND	ND	ND	ND	ND	0.49	ND
Peeling room	Copper drain covers	ND[a]	1.19	ND	1.09	1.19	ND	ND	1.79	ND	ND	ND	ND
	Control drain covers	1.53	1.27	1.27	0.49	3.19	0.79	ND	1.49	ND	ND	ND	ND
	Copper-containing coating	1.19	1.74	ND	0.49	1.09	ND	ND	ND	0.49	ND	ND	----
	Control coating	ND	1.81	ND	0.49	2.90	ND	ND	ND	ND	0.49	ND	----
	Copper containing concrete	0.79	1.27	ND	0.79	0.79	ND	ND	ND	ND	ND	1.09	ND
	Control concrete	1.19	0.97	ND	0.79	ND	ND	ND	0.97	ND	ND	1.19	ND
	Original floor	ND	0.49	ND	0.79	1.09	ND	ND	ND	ND	ND	1.49	ND
Packaging room	Copper drain covers	ND	0.79	ND	ND	ND	ND	ND	ND	0.49	ND	ND	0.49
	Control drain covers	1.44	0.97	ND	ND	1.27	0.49	0.49	ND	ND	ND	ND	ND
	Copper-containing coating	0.49	1.09	0.49	ND	0.49	ND	ND	ND	0.97	ND	ND	ND
	Control coating	0.79	1.94	ND	ND	1.33	ND	0.79	ND	ND	ND	ND	ND
	Copper containing concrete	0.79	ND	ND	ND	1.09	0.79	ND	ND	0.49	ND	ND	ND
	Control concrete	ND	ND	ND	ND	2.19	ND	0.79	ND	ND	ND	ND	ND
	Original floor	ND	1.53	1.19	ND	0.79	ND	ND	ND	ND	ND	ND	1.33
Walk-in cooler	Copper drain covers	1.27	1.19	----[b]	ND	2.09	----	ND	0.49	----	0.97	1.27	----
	Control drain covers	0.79	0.97	----	ND	ND	----	ND	0.49	----	0.49	0.79	----
	Copper-containing coating	1.44	ND	ND	ND	0.49	ND	0.79	ND	ND	0.79	ND	ND
	Control coating	0.49	1.19	ND	ND	1.79	0.97	0.49	ND	ND	0.49	0.49	ND
	Copper containing concrete	ND	1.33	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Control concrete	ND	2.27	ND	ND	0.79	ND	ND	0.97	ND	ND	ND	ND
	Original floor	ND	ND	ND	ND	1.49	ND	ND	0.97	ND	ND	ND	ND

[a] ND – non-detectable bacterial level in a sample.
[b] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

Table 2.6. Bacterial counts of *Listeria species* on copper-containing coatings and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper-containing coating	1.86	3.01	1.51	2.49	3.21	2.49	2.27	1.72	2.90	ND	4.10	----[b]
	Control coating	0.89	2.18	2.76	2.39	3.50	3.69	2.33	1.67	3.27	ND	4.09	----
Peeling room	Copper-containing coating	3.94	3.93	2.94	1.83	3.60	4.34	1.53	1.39	3.10	1.44	4.09	----
	Control coating	3.60	2.57	3.35	1.09	2.67	4.50	1.83	1.33	2.81	1.77	2.89	----
Packaging room	Copper-containing coating	ND[a]	1.69	1.27	ND	ND	1.72	3.55	ND	2.81	ND	0.97	2.97
	Control coating	ND	1.30	1.77	ND	0.49	1.53	3.19	ND	ND	0.49	0.49	4.27
Walk-in cooler	Copper-containing coating	0.97	0.49	ND	1.77	ND	0.97	ND	2.44	ND	2.31	0.49	0.79
	Control coating	0.97	3.16	ND	1.77	4.24	0.97	0.49	2.90	ND	2.27	2.19	0.79

[a] ND – non-detectable bacterial level in a sample.
[b] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

Table 2.7. Bacterial counts of *Pseudomonas species* on copper-containing coatings and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper-containing coating	ND[a]	3.49	1.49	ND	1.27	ND	ND	1.49	2.97	2.09	1.49	----[b]
	Control coating	ND	4.39	3.19	ND	3.39	3.79	ND	ND	2.97	0.97	ND	----
Peeling room	Copper-containing coating	ND	1.97	3.97	ND	0.49	3.49	ND	2.97	4.49	ND	ND	----
	Control coating	3.49	2.44	3.97	2.49	3.09	3.97	ND	1.27	4.49	3.74	ND	----
Packaging room	Copper-containing coating	0.79	2.01	1.57	ND	1.33	3.09	1.49	0.49	ND	ND	1.95	ND
	Control coating	1.83	2.69	3.79	ND	1.81	2.49	2.44	ND	ND	ND	ND	ND
Walk-in cooler	Copper-containing coating	5.06	2.19	ND	ND	0.49	ND	ND	ND	0.79	3.53	ND	ND
	Control coating	3.79	3.44	ND	2.49	2.64	ND	ND	2.97	1.09	3.95	3.27	ND

[a] ND – non-detectable bacterial level in a sample.
[b] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

Table 2.8. Total aerobic count on copper-containing coatings and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper-containing coating	2.79	5.26	3.09	4.27	5.25	4.08	1.64	5.06	4.16	2.27	6.24	----[a]
	Control coating	3.09	5.57	4.16	4.67	5.77	4.83	1.72	6.46	4.27	1.09	5.79	----
Peeling room	Copper-containing coating	7.02	4.74	4.22	6.51	4.85	6.06	3.19	4.87	5.67	2.06	5.85	----
	Control coating	6.59	4.16	6.81	4.17	4.63	6.82	1.85	0.97	5.64	5.32	5.83	----
Packaging room	Copper-containing coating	2.97	2.85	2.97	2.97	2.58	3.81	3.49	0.49	4.57	0.49	3.27	4.57
	Control coating	2.97	3.55	5.87	3.49	2.96	5.00	3.19	0.79	6.04	2.33	3.44	6.04
Walk-in cooler	Copper-containing coating	4.85	3.69	2.97	3.94	3.09	3.09	4.67	0.97	3.09	3.70	3.81	3.16
	Control coating	5.23	5.49	3.09	3.27	5.44	2.97	5.28	1.33	3.33	4.59	4.33	2.79

[a] Dash symbol ("----") indicates that the sampling was not performed because a sampling site was either initially absent or destroyed with time.

Table 2.9. Bacterial counts of *Listeria species* on copper-containing concrete patches and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper containing concrete	1.33	0.89	0.67	1.92	2.72	1.39	1.60	1.31	3.21	ND	4.02	ND
	Control concrete	ND[a]	1.72	1.94	2.67	3.19	0.49	1.44	ND	3.25	ND	4.03	ND
	Original floor	1.60	2.82	2.02	1.67	2.65	0.49	1.67	1.81	3.26	0.97	3.96	2.09
Peeling room	Copper containing concrete	2.72	4.21	0.49	2.03	1.33	3.60	1.19	1.81	3.10	0.97	3.94	4.87
	Control concrete	1.94	2.80	3.65	ND	0.67	4.48	0.97	0.97	2.97	1.39	2.99	5.49
	Original floor	3.79	4.38	4.00	ND	4.97	4.23	1.27	3.16	3.15	0.49	4.09	4.39
Packaging room	Copper containing concrete	ND	0.89	1.65	ND	ND	1.49	0.49	ND	ND	ND	ND	3.39
	Control concrete	ND	2.69	1.73	ND	ND	1.85	1.33	ND	ND	ND	ND	4.10
	Original floor	ND	4.10	ND	ND	2.74	3.19	2.64	ND	0.49	ND	1.27	3.19
Walk-in cooler	Copper containing concrete	ND	1.36	0.49	2.51	ND	2.19	0.97	ND	ND	1.34	1.97	0.79
	Control concrete	ND	3.00	ND	ND	3.39	1.09	ND	3.19	ND	2.44	0.97	0.49
	Original floor	1.90	3.97	ND	1.53	3.76	1.57	ND	3.95	ND	2.48	1.97	ND

[a] ND – non-detectable bacterial level in a sample.

Table 2.10. Bacterial counts of *Pseudomonas species* on copper-containing concrete patches and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper containing concrete	ND[a]	ND	0.79	ND	3.19	2.49	ND	ND	ND	ND	0.49	ND
	Control concrete	ND	2.58	2.79	3.49	2.79	ND	ND	1.49	ND	ND	ND	ND
	Original floor	0.49	3.27	1.19	3.49	3.49	ND	ND	ND	ND	ND	ND	4.09
Peeling room	Copper containing concrete	2.97	2.79	3.09	2.97	0.97	1.79	ND	ND	4.89	0.49	ND	ND
	Control concrete	2.79	1.09	2.49	ND	1.60	4.53	ND	ND	3.49	2.39	ND	ND
	Original floor	1.53	3.49	2.49	ND	1.92	4.60	ND	1.27	ND	1.09	ND	ND
Packaging room	Copper containing concrete	ND	0.97	2.87	ND	1.39	ND	1.19	ND	2.79	ND	ND	ND
	Control concrete	1.67	ND	2.49	ND	1.39	ND	1.79	ND	ND	ND	ND	ND
	Original floor	ND	3.33	3.79	ND	2.69	ND	ND	ND	1.79	ND	ND	1.79
Walk-in cooler	Copper containing concrete	ND	2.57	ND	ND	1.85	ND	2.09	0.49	1.97	2.44	ND	ND
	Control concrete	0.79	4.49	ND	ND	1.27	0.49	ND	4.09	1.19	1.49	1.97	ND
	Original floor	5.17	4.49	ND	3.79	1.44	ND	2.09	3.79	ND	3.77	ND	ND

[a] ND – non-detectable bacterial level in a sample.

Table 2.11. Total aerobic count on copper-containing concrete patches and their controls at three seafood processing facilities.

Processing area	Sampling sites	Log CFU/cm^2											
		Processing facility											
		Week 1			Week 2			Week 3			Week 4		
		A	B	C	A	B	C	A	B	C	A	B	C
Receiving room	Copper containing concrete	3.19	8.05	2.79	3.89	4.83	2.49	2.64	3.83	6.03	0.49	5.64	4.96
	Control concrete	3.85	7.96	3.19	4.67	5.24	3.77	2.60	3.19	5.95	1.49	5.89	4.33
	Original floor	3.98	7.44	4.87	5.02	5.30	5.27	3.67	2.97	6.08	2.39	6.50	5.85
Peeling room	Copper containing concrete	6.49	5.97	4.53	6.22	3.49	5.81	1.85	3.19	6.05	1.77	4.72	7.05
	Control concrete	5.52	6.33	4.53	3.64	5.97	6.64	1.72	0.79	6.52	3.49	4.27	7.13
	Original floor	6.68	6.89	5.08	4.09	5.95	6.25	3.49	3.69	6.10	2.16	5.97	5.72
Packaging room	Copper containing concrete	3.44	2.33	4.33	3.09	1.53	4.30	3.49	5.24	5.16	ND	2.19	4.97
	Control concrete	3.53	1.83	4.74	2.79	2.38	3.97	3.49	0.97	6.03	0.49	0.97	2.94
	Original floor	3.67	4.60	5.67	2.97	3.69	5.19	3.49	0.79	6.03	1.27	1.53	5.87
Walk-in cooler	Copper containing concrete	3.69	3.66	2.79	2.79	ND[a]	3.34	3.57	1.19	2.89	2.14	3.57	3.33
	Control concrete	3.74	5.94	3.79	2.79	4.41	0.49	4.30	4.83	3.19	4.00	4.33	3.49
	Original floor	4.01	5.94	2.49	5.81	4.27	3.19	2.97	4.39	5.05	4.69	4.79	3.18

[a] ND – non-detectable bacterial level in a sample.

VITA

Artem Sergeevich Rogovskyy was born on May 30, 1979, in Kiev, Ukraine. In the capital of Ukraine he received his high school education. In August 1996 he became a student of Faculty of Veterinary Medicine at National Agricultural University of Ukraine (NAUU, Kiev, Ukraine). Artem received his Doctor of Veterinary Medicine degree with concentration in infectious diseases in July 2001. After the graduation he worked at the Central State Laboratory of Veterinary Medicine (Kiev, Ukraine) first as an epidemiologist in Department of Harmonization of Ukrainian Legislation on Veterinary Medicine and Food to the EU standards and, then, as a toxicologist in Department of Toxicology and Chemistry. In 2003 Artem S. Rogovskyy was employed by Laboratory of Quality and Safety of Agricultural Products of NAUU as a food toxicologist. Since August 2004 he has been a graduate student of the Food Science Department at the Agricultural and Mechanical College at Louisiana State University (LSU, Baton Rouge, Louisiana) pursuing his Master of Science degree in food science with concentration in food safety and applied microbiology. Artem has been admitted to College of Veterinary Medicine at the University of Georgia (UGA, Athens, Georgia) for fall 2006 and will be pursuing a doctoral degree in Department of Infectious Diseases.