Phytosanitary treatment of the Mediterranean snails, (*Cernuella cisalpina and Eobania vermiculata*), hitchhiking with the imported tiles in packaging, using steam and vacuum

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Abstract

Alien species are being moved around the world at the unprecedented rates as a result of the increase in the international trade. Snails may be also transported from one country to another country in and on internationally traded commodities. Snails consume vegetation, but more importantly carry and spread diseases. Snails indigenous to Italy have arrived to the US on unit loads of tiles. In this study, two species of Mediterranean snails, (*Cernuella cisalpina* with weight of 0.096 g *and Eobania vermiculata* with the weight of 2.06g) were field collected in Maryland, transported to and quarantined at Virginia Tech. Unit loads of tile were inoculated with these snails and then subsequently treated with the saturated steam at the initial levels of 100, 250 and 500 mmHg. The results revealed that vacuum and steam technology can be used to kill both snails, *Eobania vermiculata* and *Cernuella cisalpine* at the temperature of 56°C with the holding time of 30 minutes in less than 61 minutes with average treating time of 51.1 minutes at the initial vacuum levels of 100 mmHg and 250 mmHg. However, it took longer than 4 hours to treat the products at 500 mmHg. There are no measureable color and shape change to either tile. The average breaking strength for ceramic tiles for both treated and control samples are 408.35 and 430.75 lbs respectively. And average breaking strengths for marble tiles for both treated and control samples are 609 and 689.5 lbs respectively. The average bending strengths of treated tiles were 5% and 12% less than that of untreated tiles respectively. For ceramic tile, it adsorbed about 0.78% moisture of total weight during treatment. There was no change in the MC of marble tiles. The corrugated paper boxes pick up about 4% moisture during treatment. For the corrugated paperboard boxes, the average Burst strengths of control and treated samples are measured to be 203.2 and 267.1 lb/in^2. The treated paper board boxes has slightly higher in the burst strength than control paperboard boxes. ECT for control and treated corrugated boxes 37.3 and 37.9 lbs respectively. The average compression strength of control and treated box samples are 407 and 375 lbs respectively. There are no significant differences between them in both box compression and ECT strengths before and after treatment. Corrugated packaging must be protected from liquid condensate during treatment.

1. Introduction

Invasive species are being introduced faster into the US because of increasing global trade. The introduced pests impact agriculture, the environment and commerce. Some of the pests can harbor human pathogens. Snail can transport liver fluke diseases. In the United States, annual costs associated with damage to the environment and to agriculture caused by alien species have been most recently estimated as US$120 billion (Pimentel et al. 2005). Much attention has been focusing on the invasive plants and insects. Mollusks also impact on agriculture (Godan 1983), biodiversity (Lydeard et al. 2004), and human health (Hollingsworth et al. 2007) and can become major public nuisances (Civeyrel and Simberloff 1996). Snails move on products and packaging. Snails can harm nursery stock, grass seed fields, vineyards, fruits and crops. Snails can carry diseases and parasites that are harmful to native animals and human.

One of the most diverse animals outside the arthropods is gastropods with more than 100,000 described species (Steinke et al., 2004). The subclass pulmonata contains the most described species with approximately 35,000 species (Solem 1984). Snails belong to the class gastropods of mollusks. Most snails are herbivores. The snail can retract its head and foot into the shell to protect the snail from predators and desiccation.

Most gastropods are aquatic, but one group have expanded into terrestrial habitats. Terrestrial snails are extremely diverse with their colors, shapes and sizes. The terrestrial snails are easily spread on plants, soil, fruits, garden materials, etc. Many European and North American species have established successfully outside of their original range. Most land snails are nocturnal in order to avoid losing moisture through the skin.

Cernuella virgata, the maritime garden snail, has caused a serious problems in Australia (Baker 2008). The snail frequently climbs to the top of vegetation to escape high temperatures and drought conditions. Toxins from snails contaminate grain and is harmful to animal or human consumption. The snail can contaminates pastures. *Cernuella virgata* is native and endemic species to Mediterranean and Western Europe including the British Isles. This species was reported in the Eastern USA (Cowie et al. 2009) and Australia (Baker 2008), where it represents an introduced invasive species. In the USA, *Cernuella virgata* is considered of priority quarantine importance (Cowie et al. 2009), while in Australia the species has the status of a serious pest species in agriculture (Baker 2008). The snails are principally regarded as pests because they interfere with grain harvests (Baker et al. 2012, Flint 1998).

According to Bean (2013), there have been approximately 12,000 mollusk interceptions at US ports, airports and border crossings in the past 10 years. Mediterranean snails usually come with the imported tiles. Since 1984, *Cernuella spp.* and *Eobania vermiculata* have been intercepted 2,722 times in the US, 55 times in Baltimore ports of entry; where 45 of these shipments were destined for sites in MD. Two gastropod exotic snails were found to in Baltimore. The company received cargo from a maritime terminal in the Port of Baltimore.

Eobania vermiculata, also called the chocolate banded snail, is already established in the USA. It is considered to represent a potentially serious threat as a pest (Bean 2013). It has been suggested that this species be given top national quarantine significance in the USA (Cowie, et. al. 2009). Officials found the species around the holding area, buildings, on trees, and railroad tracks.

Physical phytosanitation treatments include mechanical, ultrasound, vacuum and cold and hot temperatures. Mechanical methodologies include brushes and water sprays (Prusky et a1. 1999). Ultrasound generates alternating high and low pressure waves that cause cavitation at the cellular level (Hansen 2001). Heat treatments have been accepted for treating the commodities entering the USA and for interstate shipments (USDA 2005). Heat treatment is environmentally benign. Since ancient times, solar or fire heat has been used to control insect pests. The heat of the sun killed insect pests in stored grains (Cotton 1963). In more recent times, the extensive use of heat treatment has been studied and used to control grain insects and kill the insects in the wood products. In the heat treatment, heat can be generated by various methods: chemical oxidation, combustion, electrical resistance and electromagnetic exposure. The various forms in which heat is produced may affect products and the success of a given treatment depends on its ability to control insects without causing harm to the products.

Vacuum and steam treatment is fundamentally different from conventional hot air kilns. Owing vacuum steam treatment, water vapor is the medium of heat exchange rather than air used in the conventional heating system. Vacuum steam treatment is significantly faster than conventional convective hot air treatment. The pallets were treated in less than 65 minutes that included the vacuum time of 5 minutes and the holding time of 30 minutes (Chen et. al. 2012).

The steam heat can effectively penetrate logs and raise the internal temperatures to kill the pests without causing damage to logs that could significantly affect the veneer yield and values (Chen et. al. 2012). In general, heat treatments do not have any significant deleterious effects on log quality.

While there has been much research regarding the heat treatment of insects, no much research was found describing the heat treatment of snails. It is goal of this study to determine the efficiency of steam and vacuum to treat quarantine tile in its original packaging.

2. Objectives

Specific project objectives,

To evaluate the effectiveness of using steam and vacuum to kill snails transported in unit loads of package tile.

To recommend the effective steam vacuum treatment schedule for the treatment of snails.

To determine the effect of the steam and vacuum treatment of unit loads on the performance of the packaging.

To determine the effect of the steam and vacuum treatment on strength, warpage and color of tile products, including marble and ceramic tiles.

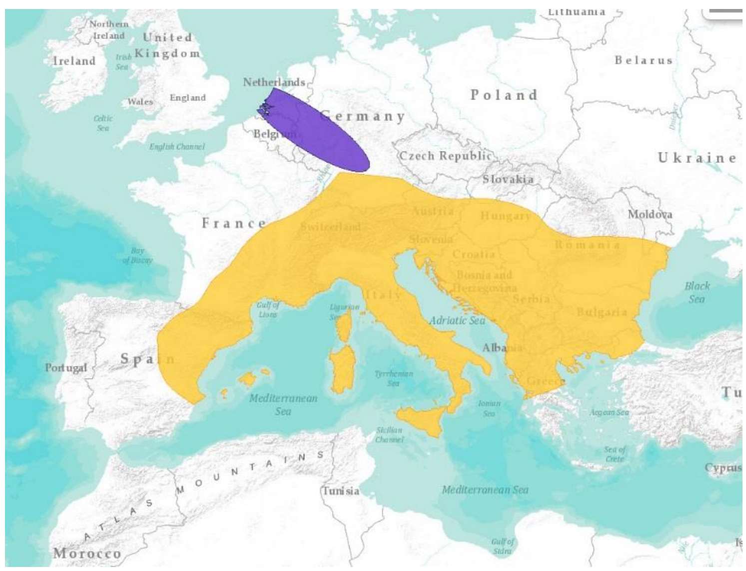
3. Testing quarantine snails

Species description

Two invasive species introduced from Mediterranean were tested in this study. Both species are air-breathing and terrestrial snails. The tiles, from the Mediterranean region, are known commodities that commonly transport these two species.

Cernuella cisalpine, a maritime garden snail, has been found in coastal Virginia, Maryland and North Carolina. This small snail has a white and brown striped shell. It is natively to Italy (Figure 1). It has spread to Germany, Belgium and the Netherlands. In Europe, this species is mostly distributed by cars (Figure 2).

Figure 1. The *Cernuella cisalpine*, growing in Baltimore, Maryland.



Source: http://maps.iucnredlist.org/map.html?id=156398

Figure 2. The distribution of *Cernuella cisalpina* in the Europe.

Eobania vermiculata is another European snail that has been introduced to US. The color of the shell is whitish to greenish yellow, often with multiple color bands or spots; the lower side is frequently with two brown bands and whitish between lowest band and umbilicus. It gets another name of chocolate banded snail. The shell has 4-4.5 whorls (Figure 3). The snails can be caught because they will aggregate together under tarp covering the grassy areas. The tarp was flipped and snails can be found (Figure 3). The width of the shell is 22–32 mm and the height is 14–24 mm (Texas Invasive Species Institute, n.d.). *Eobania vermiculata* can live up to 5 years to reach maximum size (33 mm). The chocolate banded snail is used as food in many countries due to its larger size (Figure 4).

Figure 3. Chocolate banded snails living under the tarp alive.

Figure 4. *Eobania vermiculata* snail crawling on the Tupperwear

Vineyards are very threatened by *Eobania vermiculata,* being observed on grape plants in their native range. That makes this snail a direct threat to grape growers in California and Texas (Texas Invasive Species Institute, n.d.). Snails tend to feed on a variety of items found in their natural habitat. Some common items for their diet include plants, fruits, vegetables, and algae. Plants that are decaying are often a good meal for them. Snails get some calcium from dirt.

Collecting the snails and transporting the quarantine snails

The naturalized snail species (*Cernuella cisalpine and Eobania vermiculata*) were field collected from (8410, Beachwood, Baltimore, MD 21222 and 2293 South Clinton Street Baltimore MD 21224) with Maryland Department of Agriculture staff.

Figure 5. In the Baltimore, where the snails were found and collected.

The snails live on the grassy locations outside the holding area and near the fences. The ground was dry (Figure 6). Snails were found near the fence, on the fence and on the track and even hanging on the aluminum pole (Figures 7 to 13).

Figure 6. Snails were found on dry soil and in grassy areas.

Figure 7. A snail picked up near the fence.

Figure 8. A snail was hanging on the fence.

Figure 9. A snail was climbing and sticking on the surface of the aluminum pole.

Figure 10. A snail was found on the tall bushes.

Figure 11. A snail was found under the railway track.

Figure 12. The snails usually were found near the holding area.

Figure 13. The soils where the snails were living.

The snails were hand-picked and placed carefully in special containers and sealed. Sod was placed on the bottom of container to keep the snails alive (Figure 14). These containers were locked as an added precaution. The containers with snails, were placed in a vehicle at the Maryland Department of Agriculture headquarters and transported back to Virginia Tech. The containers were stored in a quarantine laboratory located at Virginia Tech. The door of the quarantine lab was secured.

Figure 14. The snails were kept alive in the sod.

Transporting containers.

Two larger transport containers (Figures 15-16) and several Tupperware containers (Figure 17) were purchased to contain and transport the snails from quarantine lab to the treating facility. Transport containers were constructed to prevent escape (Figure 15). The size of container is 23 ×14.5×13.5 inches. The containers have a cover constructed of screen (mesh size 1/8 inch or smaller). The rear containers were reconstructed coolers. The lid is secured by a latch and lock that prevents accidental opening (Figure 16).

Figure 15. Special transporting container used to move the snails.

Figure 16. APHIS Inspector examined the transporting container for security of the snails.

Figure 17. Tupperware was stretch wrapped with the snails inside to prevent their escape.

The container has a 3 – inch wide copper strip that encircles the container (Figure 18). There is a space of more than 8 inches left between the soil inside the container and the lid. The copper strip can prevent the snails from crawling out of the container. Copper has a components that are micro-biocidal. The copper ion on thiol enzymes and possibly other thiol groups are poisonous to microbial cells. The copper metal inhibits microbial growth. Copper compounds are also used in the wood, paper and paint industries as preservatives.

Figure 18. Copper strip encircles the walls of the container to keep the snails from escaping.

Kentucky bluegrass from turf research laboratory at Virginia Tech was collected (Figure 19). This turf was free from any herbicides. Sods were laid on the container as a food resource and habitat for snails (Figure 20).

Figure 19. The snails were transported back to Virginia Tech and kept in quarantine facilities on Price Fork Road, in Blacksburg, Virginia.

Figure 20. The turf were placed inside the container.

4. Treating facilities, equipment and materials

Insect quarantine laboratory at Virginia Tech.

The Virginia Tech Beneficial Insects Laboratory (Figure 21) was certified as a containment facility by APHIS in 1972. It is located at the Prices Fork Rd. This containment facility laboratory is under the control of the Department of Entomology of Virginia Tech. This laboratory is used to screen arthropods as potential biological control agents of noxious weeds and arthropod pests. This includes host specificity testing of exotic beneficial insects and determination of their basic biology before their introduction into the United States.

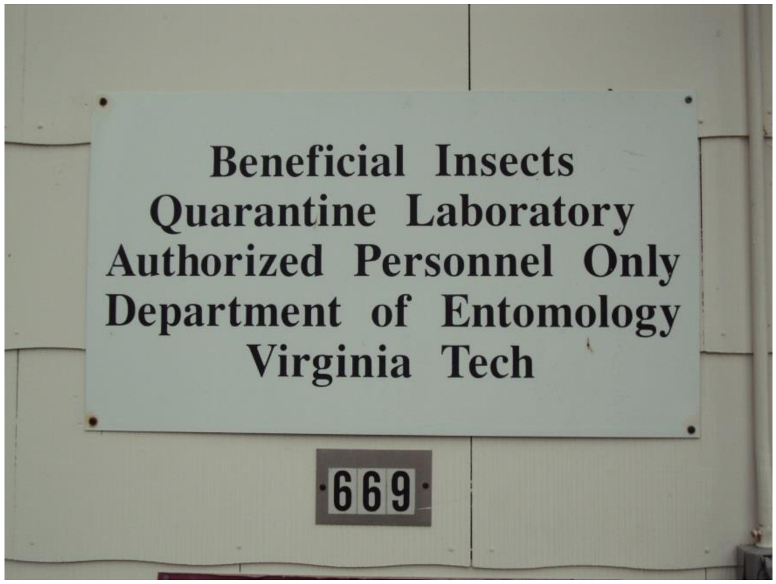


Figure 21. Virginia Tech Beneficial Insects Laboratory

In this facility there is maximum security room, rearing rooms, and a walk-in cooler with interior and exterior steel walls and 4 inches of foam insulation. The floor is concrete. All windows, drains, vents and other openings in the facility (except the entrance door) are covered with one-eightth inch mesh or hardware cloth. All cracks and crevices are all sealed. The only door is the entrance door and is self-closing.

With the security concerns, the applicant and persons designated were allowed entry to facility and the facility is locked at all times when applicants are not present. The waste that was in contact with snails was sterilized in the autoclave before disposal and poured down the drain that flows into a septic drain field system.

The material to be autoclaved is double wrapped in sterilizable bags before sterilizing. All waste was sterilized in a Tuttnauer™ autoclave at 250^{o}F for 45 minutes at 30 psi. The autoclave is inspected and serviced monthly by a university physical plant or departmental technician.

All materials except soil that are removed from the containers (dead snails and other debris) is immersed in a bleach solution for 5 minutes before disposal. All soil removed from the containers was sterilized by steam heat with pressure up to 15 lbs then maintain temperature at 250 F for 30 minutes.

Trailer

An enclosed cargo trailer with internal dimension of 8.5 by 20 by 7.5 feet in height was used to house the vacuum chamber (Figure 22). It has double rear doors. The opening is 88 inches wide by 84 inches height. There is a side door of 32 inches wide. Two axles of 5200 lbs are designed to support the heavy weight of chamber and tiles, or other loads (Figure 23). Four 15 inches tires on the lug wheels can support up to 8000 lbs. The side wall is 3/8 thick plywood with an aluminum exteriors. There is a roof vent.

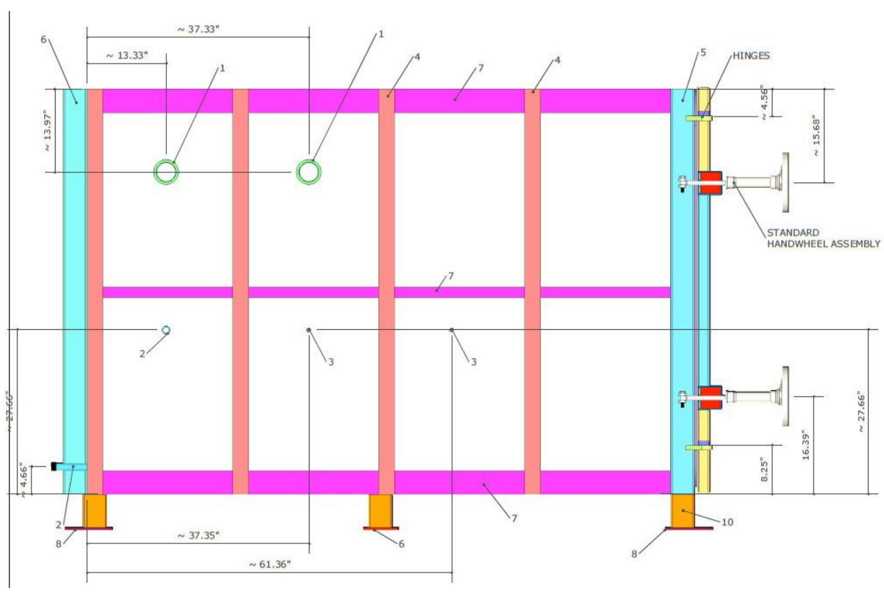
This trailer can easily transport the vacuum steam system to other locations.

Figure 22. The twenty feet trailer that was used to transport the treating system.

Figure 23. Mobile treating facility holds the vacuum chamber, boiler, vacuum pump and other equipment.

Vacuum Chamber

The stainless steel vacuum chamber was custom manufactured by the Vacutherm (Figure 24). The inside dimension of chamber is 5ft (W) x 5ft (H) x 8.5ft (L). The chamber is steel reinforcement to resist the force induced by the vacuum (Figure 25). Vacuum level can be as low as to 0 pressure. Plumbing connections pass through wall to allow the air and steam to flow in and out. The vacuum chamber has a swinging door that is well sealed with the rubber.



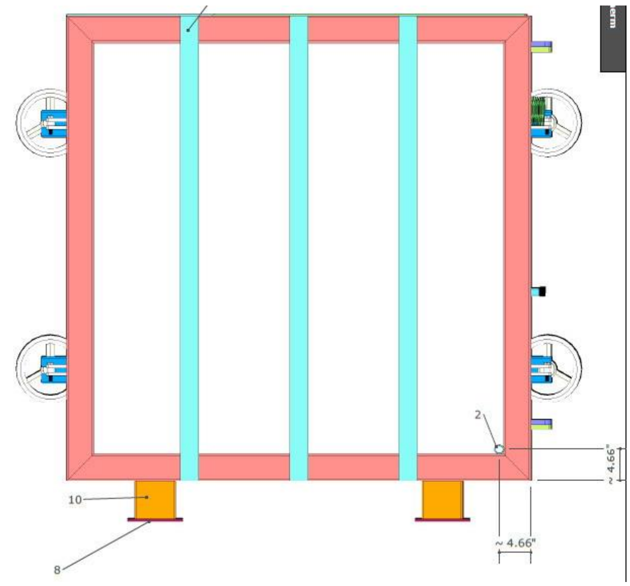


Figure 24. The mechanic drawing of vacuum chamber.

Figure 25. The 5ft (W) x 5ft (H) x 8.5ft (L) vacuum chamber was custom built and used in this study.

Electric steam generator

The electric steam generator (Reimers Electra Steam Inc. Model RX100C3F, 208V/360) was equipped with heating elements of 100 KW, 10 BHP. It can produce 342 lb/hr of steam at 212° F. The boiler has adjustable pressure control and manual reset hi-limit temperature control, heavy duty magnetic contactors, and a combination low water cut-off. It is equipped with the solenoid valve, strainer, pressure gauge, ASME safety valve, check valve, blowdown valve, steam outlet valve and water gauge glass (Figure 26).

A boiler blowdown tank was not installed in the system because boiler will not be used continuously for a long period of time. Boiler blowdown is water intentionally wasted from a boiler to avoid concentration of impurities during continuing evaporation of steam. The water is blown out of the boiler with some force by steam pressure within the boiler. The steam boiler evaporates steam from liquid water; and requires frequent replenishment of boiler feedwater for the continuous production of steam required by most boiler applications.

Figure 26. The electric steam boiler is equipped with 100 kw heating elements.

Vacuum pump

The Mink vacuum pump (MI 2122) is a 7.5 HP, two-stage, dry claw, pump (Figure 27). The pump can pull a vacuum to 8 mm Hg with a capacity of 56 cfm at atmospheric pressure. The Mink vacuum pump works according to the rotary lobe principle. As the lobes rotate constantly, water vapor and air are pulled in, compressed and discharged under pressure.

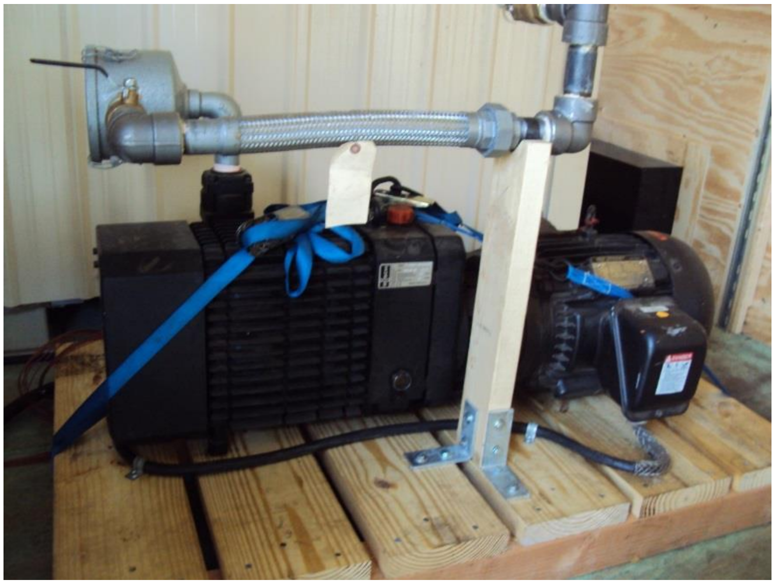


Figure 27. Busch Mink 7 horse power vacuum pump can remove about 56 cfm of air.

Testing tiles

Both ceramic (45 by 45 by 1cm thick) and marble tiles (30.5 by 30.5 by 1 cm thick) were used in this study (Figure 28). Ceramic tile is a mixture of clays which have been shaped and fired at extremely high temperatures. Its density also determines the strength of the bisque as it relates to the water absorption level. The strongest bisques (those suited for heavy commercial installations) have the smallest and fewest number of air pockets which, in turn, will affect the all water absorption, breaking strength, and impact resistance of the finished product. The density of the clay also determines if the tile is or is not suitable for outdoor use.

Figure 28. The ceramic tiles imported from Italy that were used in the test

Marble tiles (Figure 29) have a beautiful, unique look with all their whirling patterns and shade variations.

Figure 29.   Marble tile imported from Italy was used in the test

Corrugated paper board test

During the vacuum steam treatment, the brown corrugated paper boards were laid on the deckboard of pallets and on the top of unit load of tiles to check the effect of treatment on paper board quality, such as, moisture pick up, the burst strength and ECT of paper (Figure 30).   Five tests on the brown corrugated paper boards were performed at both 250 and 500 mmHg vacuum levels (Figure 31).

Figure 30. The corrugated paper boards were placed on the top of unit load of tiles.

Figure 31. The corrugated paper boards after being treated.

Packaging corrugated paper board boxes

Forty eight corrugated boxes were made in the lab with the size of 6 by 6 by 12 inches (Figure 32). They were shrink-wrapped with the six boxes at the each layer and two layer of 12 boxes. This way, the adsorption of moisture during the vacuum steam treatment can be studied to determine the effect of treatment on box compression strength (Figure 33). During treatment, the opening of shrink wrap was facing down as that was in the tile packaging (Figure 34). This simulated how the packaged tile was covered with shrink film.

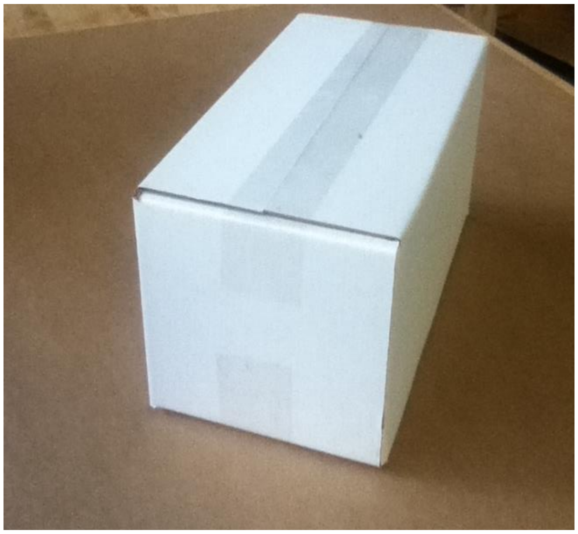


Figure 32. The corrugated paper box 6 by 6 by 12 inches was made and used to simulate the effect of treatment on box comparison.

Figure 33. The corrugated boxes were stacked together the same way as the tile packaging boxes in the unit load.

Figure 34. The corrugated paper board boxes were then shrink-wrapped.

The corrugated paper boxes were labeled so that it could be determined how box location may affect water adsorption. Two layer of 12 boxes were arranged, with 6 of them in each layer.

5. Testing procedure

The vacuum steam chamber in the trailer was located just outside the Phytosanitation Laboratory on pavement. The unit load of tiles supported with a pallet was loaded into the container (Figure 35). To limit condensation from the roof of the chamber falling onto the unit load of tiles, a roof structure was built over the unit load. This is shown in Figure 35.

Figure 35. The unit load of tiles was set on two aluminum pallets inside the chamber.

In preparation for the experiment, the small Tupperwares plastic containers were used to transport the snails from the quarantine lab (Figure 17). These containers were opened individually, only as required on a work table in the Phytosanitation Laboratory. Each snail was weighed before and after treatment. It is interesting to check the weight change of snails after treatment during the tests.

Small packets were made from cheese cloth (Figure 36). Five *Cernuella cisalpina* snails or three *Eobania vermiculata* snails were placed in each packet. Packets were then labeled to identify the locations within the unit load (Figure 37). Packets with snails were placed at the various locations in the unit load of the tile. The packets provided the added containment while allowing for easy treatment, extraction and secured collection post-test.

Figure 36. The small packets made from cheesecloth to contain the snails.

The small packets of contained snails were placed in the palletized tiles. At the same time, there is at least ten snails of each species that were set aside as control samples during the test. Once a test has been completed, the snail packets were carefully removed from the palletized tile and placed in an empty Tupperware container. The Tupperware container were opened on the work table and individual snail packets were removed and examined under a light microscope to detect heartbeat as an assay for mortality. Once a packet of snails has been examined, all snails, live or dead, were placed in the Tupperware container and returned to the quarantined laboratory for disposal. The total number of snails used in the test are listed in Table 1.

The locations of the packets within the unit load are shown in Figure 38. Thermocouples were also placed at those locations to record the local temperatures. When all locations reached 56°C, the temperature was held for 30 minutes.

Eight thermocouples were arranged and placed at those locations where the packets of snails were located. At two locations, the snail packets were actually inserted between and on boxes of tiles. Shims were used to make openings for the thermocouples and the packets of snails to be inserted into the unit load (Figure 37). Other locations were the three locations on the top of unit load, three on the side of the unit load and one on a deck board of supporting pallet and another one in under the deck board (Figure 38). All of the temperatures were recorded with the computer using a data acquisition system (Figure 39).

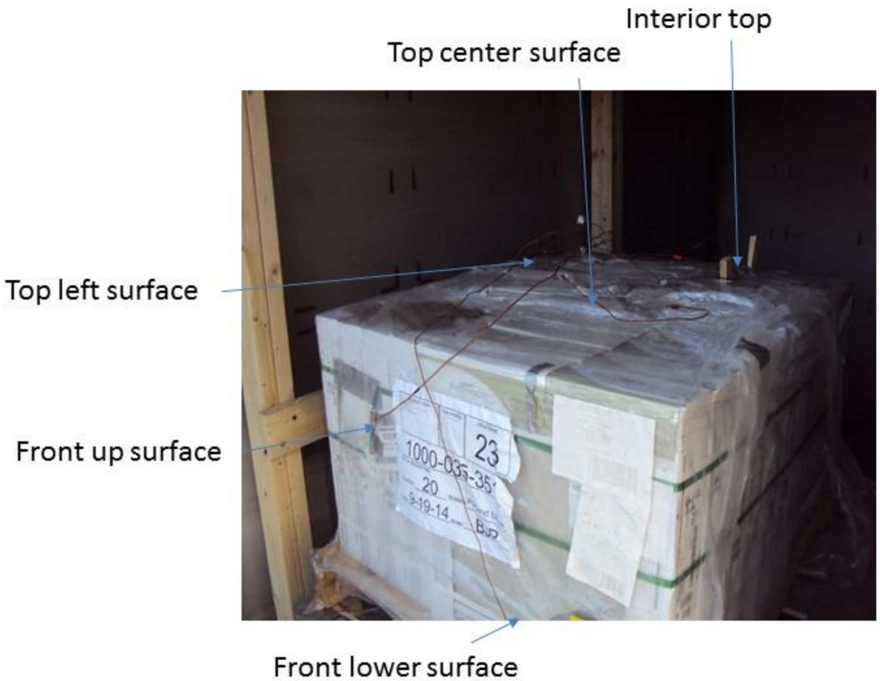


Figure 37. Unit load of tiles and thermocouples arrangement during the test.

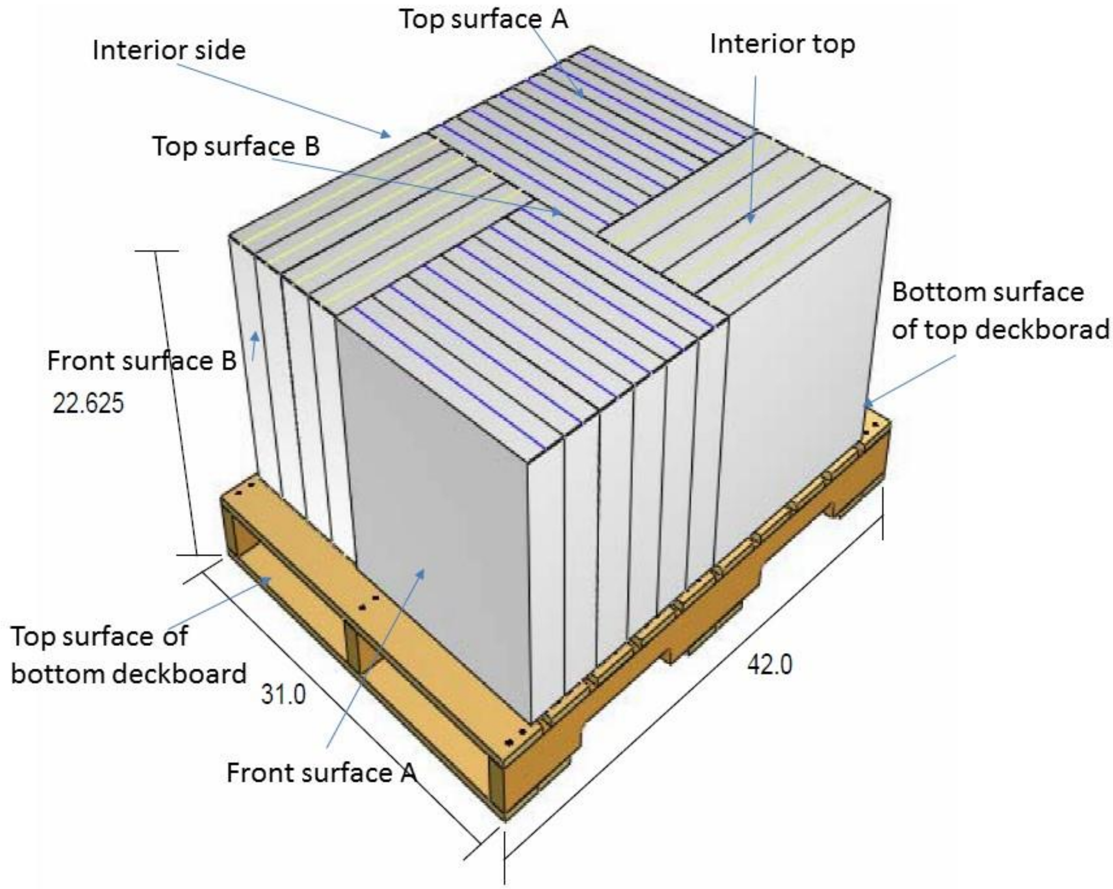


Figure 38. The location where packet of snails was inserted into the opening.

At each test, one tile was laid next to unit load to allow measurement of the weight change and later for testing of the tile strength to see any change in the strength due to the treatment (Figure 40). After the snails and tiles were placed inside the chamber, the doors were closed and chamber sealed. Vacuum is created by pump to the target pressure. When pressure inside the chamber reached the target level, pump was stopped. Steam is injected into the chamber and temperature increases. Various initial vacuum levels were used in this study.

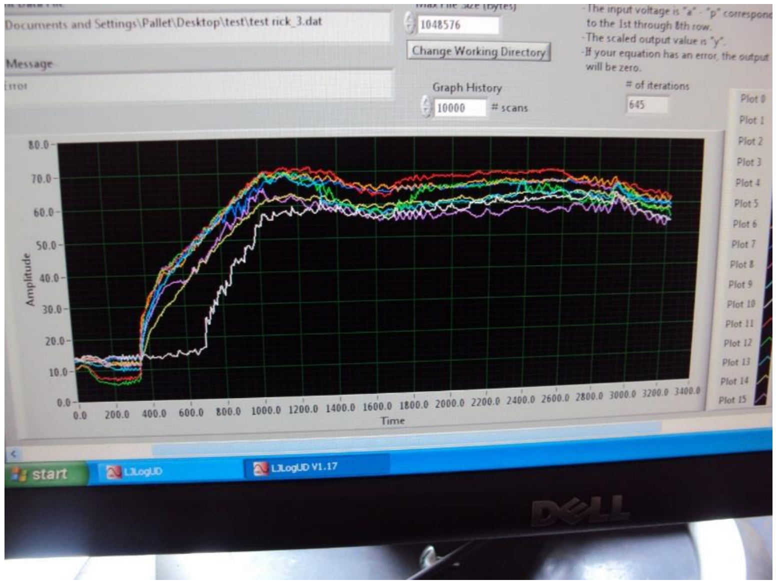


Figure 39. Example of temperature profile collected by the data acquisition system.

Figure 40. A ceramic tile was placed next to the unit load during the vacuum and steam treatment for later testing of properties.

After the prescribed holding time at the target temperature of 56°C, and vacuum was relieved inside the chamber. Condensate on the floor was discharged through a screened drain that collected any solid residual from the treatment. The chamber door was then opened. The packets on the unit load of tiles were retrieved and snails were placed at the petri dishes for examination to determine mortality (Figure 41).

Table 1. Experimental design for the tile tests.

Test Number	Tile	Tile Number	Treating Pressure (mmHg)	Snail Species	Number of snails	Burst/ECT
1	Ceramic Tile	1	250	*Cernuella cisalpina*	40	Brown paper
2		2		*Eobania vermiculata*	24	Brown Paper
3		3		*Cernuella cisalpina*	40	Brown Paper
4		4		*Cernuella cisalpina*	40	Brown Paper
5		5		*Eobania vermiculata*	24	
6		6		*Cernuella cisalpina*	40	
7		7		*Eobania vermiculata*	24	
8		8		*Cernuella cisalpina*	40	
9		9		*Eobania vermiculata*	24	
10		10		*Eobania vermiculata*	24	
11		11	500	*Cernuella cisalpina*	40	Brown Paper
12		12			40	
13		13	100		30	12 boxes
14		14			30	12 boxes
15		15			30	12 boxes
16		21			30	
17		22			30	
18		23			30	
19		24			30	
20		25			30	
16	Marble Tile	101			30	
17		102			30	
18		103			30	
19		104			30	
20		105			30	
Total					790	

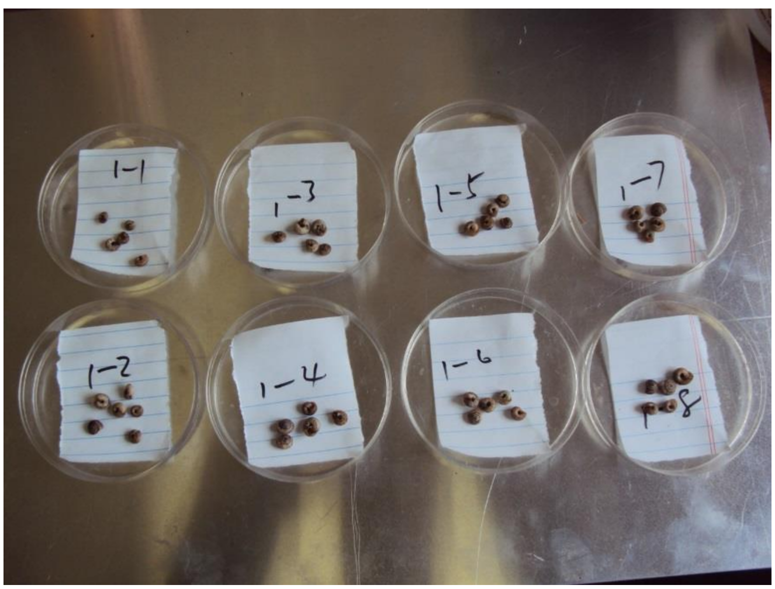


Figure 41. *Cernuella cisalpina* snails were placed in the petri dishes after treatment.

The snails’ weights before and after treatment, were measured to 1/1000 g (Figure 42).

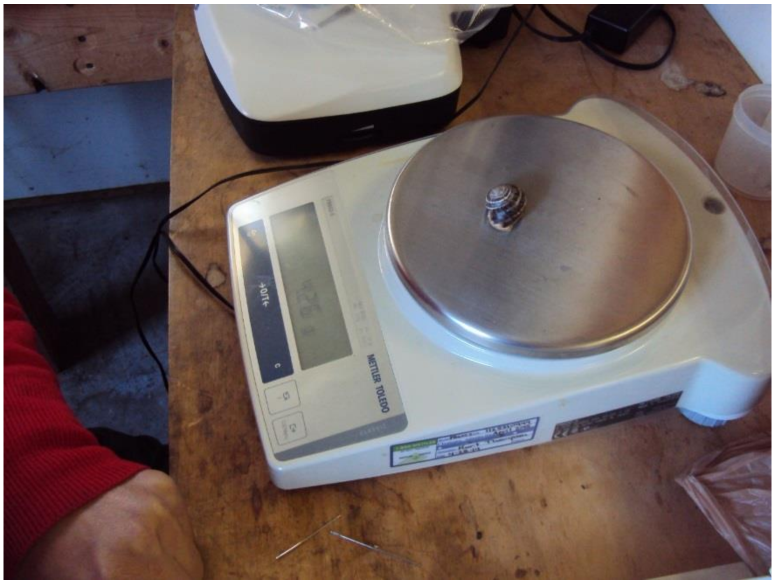


Figure 42. The scale used to weigh snails and packaging materials before and after treatment

All Tupperware containers used for transport and hold the snails, along with used cheesecloth packet material, were autoclaved after they had been used. The autoclave treatment schedule is 121°C for 40 minutes.

Snail mortality

Snail mortality was determined by either of two methods. If the heart of the snails was visible with the light microscope shown in Figures 43 and 44, then the heart was checked for beating. If the heart was not visible, the exposed foot of snail would be poked with a probe to determine if any reaction occurs as shown if Figure 45.   The snails were rechecked for signs of life after at least two days.

Figure 43. Motic light microscope (Motic SMC – 140) used to check snails for a heart beat

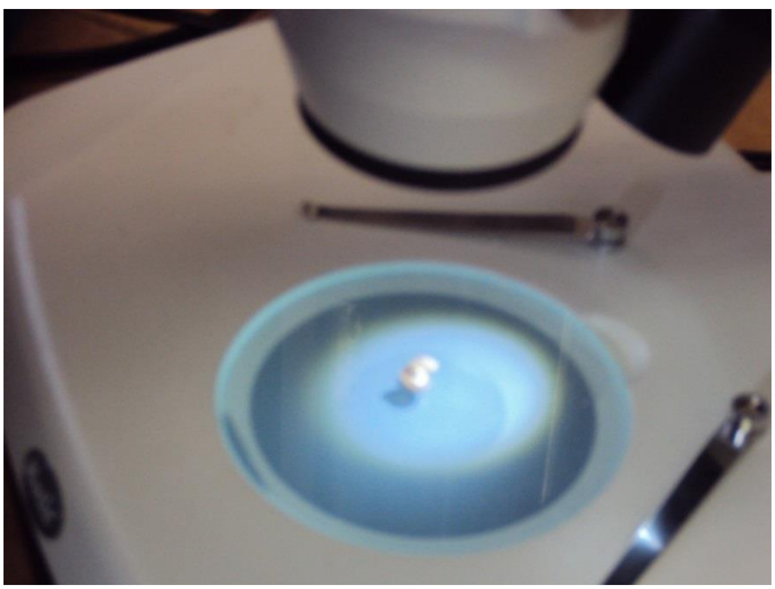


Figure 44. A snail was checked under the microscope for heart movement.

Figure 45. A needle was used to scratch the exposed foot of the snail.

Tile warpage test

To determine if the treatment affects the shape or geometry of the tile, a test of flatness was performed according to ASTM Standard Test Method for Measuring Warpage of Ceramic Tile (ASTM C485 – 09). Warpage is the curvature of a flat tile measured as deviation of the tile surface from a plane along the edges or the diagonals. The deviation is measured at the mid-length of an edge or diagonal, expressed as a percentage of the length of the edge or diagonal, and called convex or concave with respect to the face of the tile. This test method consists of measuring the deviation from a straight line at the midpoint between reference points on the face or back of a tile. Measurements are made along the edges of a tile and along the diagonals. The reference plate has been placed. A piece of tile was inserted with its face resting upon the three reference pins and its edges in contact with the three registry stops. The two dial indicators were read. We turned the tile 90° clockwise, and repeated the procedure until all four sides and the two diagonals have been measured. The distance between reference pins are 11.0 inches for the side and 15.6 inches for diagonal.

Tile breaking strength test.

In order to determine the effect of the treatment on the strength of the tile, a strength test was performed according to ASTM C648 – 04 Standard Test Method for Breaking Strength of

Ceramic Tile. This test method concerns the determination of the bending strength of ceramic tile. The test method consists of supporting the tile on the ends of three cylindrical rods arranged as an equilateral triangle, and applying force at a certain rate to the center of the tile, until the specimen breaks. The testing machine model used in this study is MTS 322.31 (Material Testing System) (Figure 46).

Figure 46. Material testing system.

The tile was placed on the steel support block (Figure 48) into the testing machine so that the load applicator is directly above the center of the steel block (Figure 46). The support shown in Figure 48 represents the larger support triangle requested by the test standard. The force at the rate of 1100 lb/inch and (3600 to 4900 N)/min was applied until the tile actually breaks into pieces. The forces were recorded in lbs.

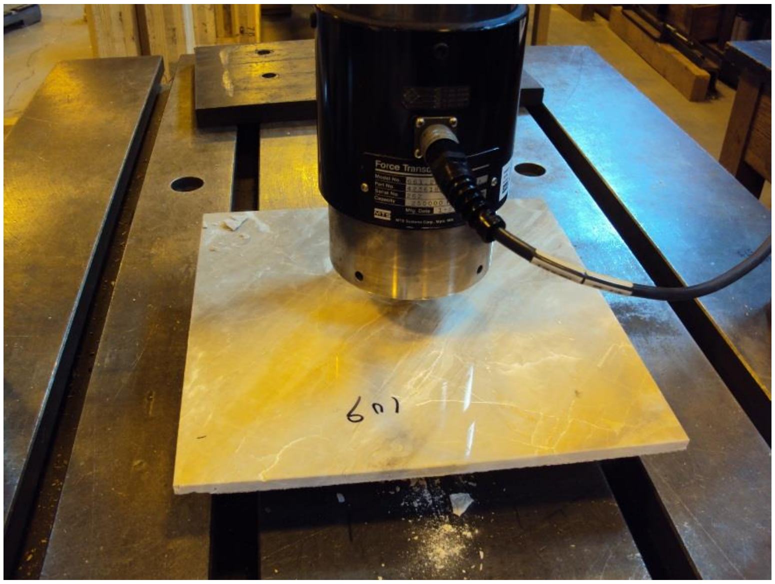


Figure 47. The tile breaking strength test.

The test machine had a load capacity of 50,000 lbs.   The equal-lateral triangular support is used in the testing. The length of sides is 3.0 inches (7.63 cm) (Figure 48).

Figure 48. The equal-lateral triangular support made in the breaking strength test.

Bursting strength test, edge crash test (ECT) and box compression test

The corrugated paper board and paperboard boxes were conditioned for more than 72 hours before they were tested for box compression, and board burst strength and ECT. Conditioning atmosphere was kept at the constant relative humidity of 50.0±2.0% and temperature of 23.0±1.0 ℃ (Figure 49).

Figure 49. The conditioning chamber.

There are currently two tests widely used by the corrugated industry to determine the strength of corrugated paperboard. The first is the Burst test also called Mullen Burst test using Mullen Testers manufactured by Stondex company (Figure 50). The Bursting test is a measure of the force required to rupture the face of corrugated board (ASTM D774/D774M – 97). This force is indirectly related to a carton's ability to withstand external or internal forces and thus to contain and protect a product during shipment. Burst strength is reported in lbs.

The second test is ECT (ASTM D5639/D5639M – 11). It is related to the stacking strength of a paper board boxes. ECT is a measure of the edgewise compressive strength of corrugated board (Figure 51). It is measured by compressing a small segment of board on edge between two rigid plates parallel to the direction of the flutes until a peak load is established. This is measured in pounds per lineal inch of load bearing edge (lb/in). The compression testing machine manufactured by Lansmont Corporation was used to perform the ECT and box compression test. The test apparatus is shown in Figure 51. A test jig for ECT measurement is shown in Figure 51.

Figure 50. Burst test is done on the Mullen tester.

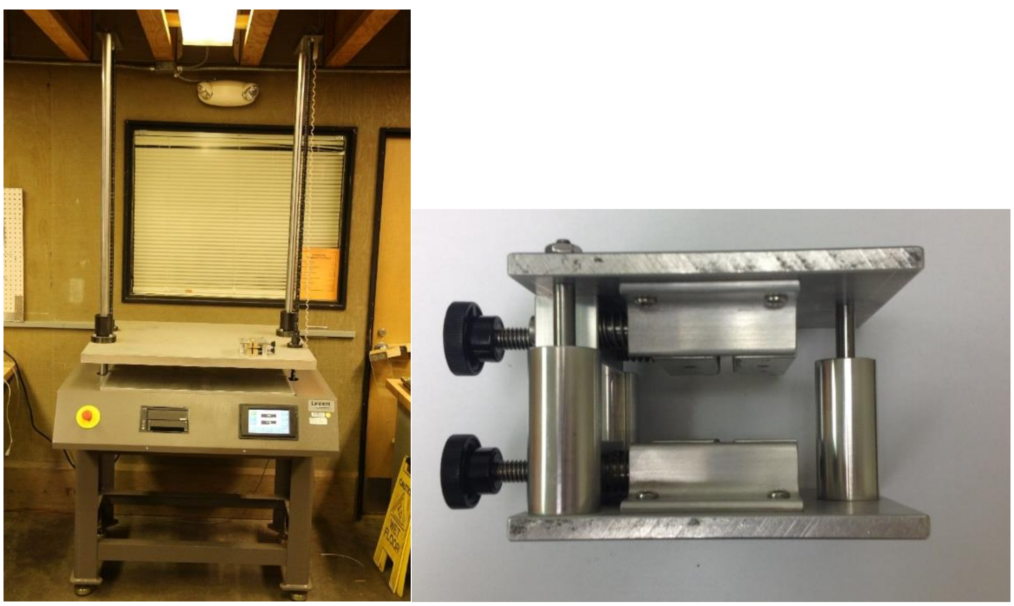


Figure 51. ECT and compression test instrument showing tester and test jig.

6. Testing result and discussion

Treating duration

Table 2 contains the treating duration of each test. Mediterranean snails, *(Cernuella cisalpina and Eobania vermiculata)*, were killed using vacuum and steam treatment with average treatment cycle duration of less than one hour at an initial vacuum level of 250 mmHg and 100 mmHg (Table 4). In the test, six hundred forty snails were killed during the tests and 96 of them are *Eobania vermiculata*. The treating duration included the average vacuum time 7.2 minutes and holding time 30 minutes. The temperature reached 56°C and was held for 30 minutes during treatment. This schedule is the ISPM15 to treat wood pallets and containers. From the test results, the HT criteria of 56°C/30 min will kill the snails in the tiles. In our experiments, the temperatures were measured on the surface of the packaged tile rather than inside the packaged tiles. It is not necessary to raise the temperature inside the products because snails can't enter between the individual tiles. They always are found in the crevices and the gaps between the packages. The steam can easily penetrate to those locations and heat up the space within the unit load between packages. At the same time, the control snails of both species were alive.

Table 2. The vacuum steam treatment duration for both snail species and tiles with holding time 30 minutes at 56°C.

Test Number	Tile	Tile Number	Treating Pressure (mmHg)	Vacuum time (min)	Steaming time (min)	Total treating duration (min)
1	Ceramic Tile	1	250	6	10	46
2		2		5	17	52
3		3		8	10	48
4		4		5	11	46
5		5		5	10	45
6		6		4	11	45
7		7		4	15	49
8		8		4	14	48
9		9		5	16	51
10		10		4	15	49
11		11	500	2	135	167
12		12		2	168	200
13		13	100	12	18	60
14		14		12	19	61

15		15		10	10	50
16	Marble Tile	101		9	16	55
17		102		9	14	53
18		103		8	15	53
19		104		10	15	55
20		105		9	15	54
Control	Ceramic Tile	31	Not treated			
		32				
		33				
		34				
	Marble Tile	106				
		107				
		108				
		109				

At atmospheric pressure of 760 mmHg (1013 mbar), water boils at 100ºC. 419 KJ of energy is required to heat 1 kg of water from 0ºC to the saturation temperature 100ºC. Another 2,257 KJ of energy is required to evaporate the 1 kg of water at 100ºC to steam at 100ºC. A significant feature of the vaporization phase change of water is the large change in volume that accompanies it. By comparing that to the volume of the liquid water, the volume expands by a factor of 1700 when vaporized into steam at 100°C. The large energy in the steam can raise the temperature in the air among tiles quickly. Typical temperature profiles are shown in Figures 52 to 54. Clearly the high vacuum is more effective in distributing heat. The water vapor condenses and releases a relatively large amount of energy to increase the temperature. This procedure is effective to kill the snails that dwell in the void areas inside the packaging materials rather than in the materials. It can be seen from Table 4 that it took about 15.3 and 12.9 minutes for initial vacuum of 100 or 250 mmHg to increase the temperature to 56°C.

Using the initial vacuum of 100 mmHg or 250 Hg, the average time to kill snails is 55.1 and 47.9 minutes (Table 3). However, using 500 mmHg, it took 183 minutes (Table 3) although the vacuum time is only 2 minutes. That means that it took longer for temperatures to reach 56°C. Also, the corrugated packaging boxes adsorbed more water and the packaging integrity was significantly affected. This vacuum level is not recommended. Some experiments were prepared in the winter time when the initial temperature of unit load of tiles was relatively low about 6°C. It may take less time to treat the materials in summer months.

Table 3. The average vacuum steam treatment duration for both snail species and both tiles with holding time 30 minutes at 56°C.

Treating Pressure (mmHg)	Average vacuum time (min)	Standard deviation	Average steaming time (min)	Standard deviation	Average total treating duration (min)	Standard deviation
100	9.9	1.46	15.3	2.71	55.1	3.68
250	5.0	1.25	12.9	2.77	47.9	2.42
500	2.0	0	247.0	55.2	279.0	55.2

Temperature profile

The temperature profile at each test was recorded for treatment. Figures 52 to 54 represent the treatment at the three initial vacuum levels. All profile curves are shown in the appendix.

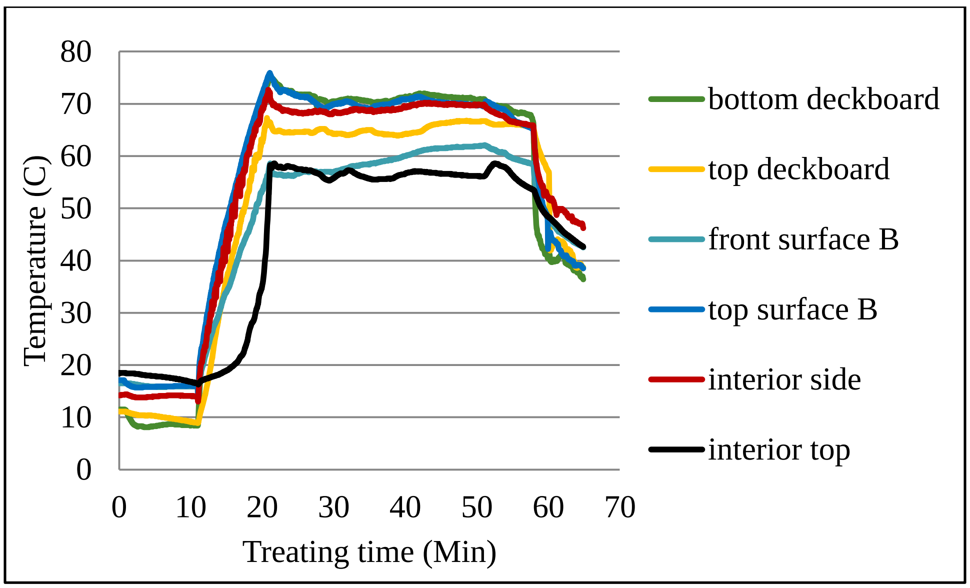


Figure 52. Typical temperature profile at the initial vacuum pressure of 100 mmHg.

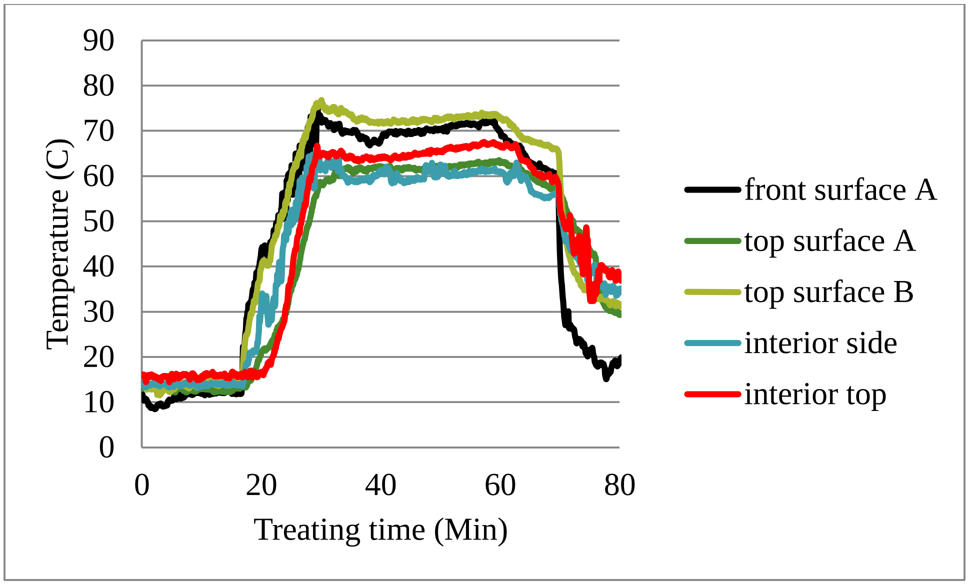


Figure 53. Typical temperature profile at the initial vacuum pressure of 250 mmHg.

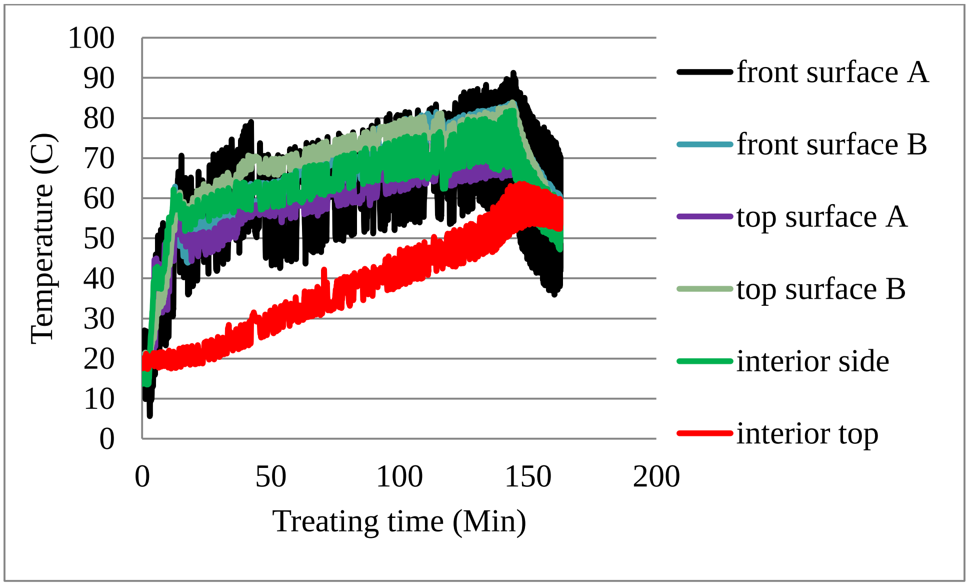


Figure 54. Typical temperature profile at the initial vacuum pressure of 500 mmHg.

Snail weight and its change during treatment.

Eobania vermiculata is relatively larger than *Cernuella cisalpina*. The average weight of the *Eobania vermiculata* in test 9 were 2.06g before treatment. The weights of the *Cernuella* *cisalpina* ranges from on 0.04 to 0.16g with average weight of 0.096g before treatment. The weights of snails were measured for *Eobania vermiculata* after treatment. Average snail lost 18.9% of its body weight. According to Fagbuaro et al. (2006), water was on average of 77% and 79% of the body weight of *Archachatina marginata (ovum) Pfeiffer* and *Limicolaria spp.* respectively. It is probable that heat rather than desiccation in the case of snail death.

Weight change and color comparison of tiles after treatment.

Weights of the treated ceramic tile and marble tile were measured and compared before and after treatment. Tables 4 contains the tile weights before and after treatment. Average weights of a piece of ceramic tile and marble tile are 3847.6 and 2329.0 grams before treatment respectively. After test, average weight changed to 3877.5 and 2329.4 respectively (Table 5). There are average increases in moisture of 0.78% and 0.02% for ceramic and marble tiles respectively. The tiles did not pick up much moisture during the test. However, the ceramic tiles picked up some moisture. It almost is zero increase for marble tiles. The color of tile products before and after treatment was compared and no change was observed.

Table 4. Ceramic and marble tile weight changes before and after test.

Test number	Tile	Tile number	Treating pressure (mmHg)	Weight before the test (g)	Weight after the test (g)	Difference (g)	Percent change (%)
1	Ceramic Tile	1	250	3889	3921	32	0.82
2		2		3796	3836	40	1.05
3		3		3888	3916	28	0.72
4		4		3855	3880	25	0.65
5		5		3859	3894	35	0.91
6		6		3801	3820	19	0.50
7		7		3851	3872	21	0.55
8		8		3854	3877	23	0.60
9		9		3878	3901	23	0.59
10		10		3865	3887	22	0.57
11		11	500	3871	3897	26	0.67
12		12		3821	3858	37	0.97
13		13	100	3871	3910	39	1.01
14		14		3882	3931	49	1.26
15		15		3789	3821	32	0.84

16		21		3798	3829	31	0.82
17		22		3885	3913	28	0.72
18		23		3860	3889	29	0.75
19		24		3833	3861	28	0.73
20		25		3806	3836	30	0.79
Average for ceramic tiles				3847.6	3877.5	29.9	0.78
16	Marble Tile	101		2310	2312	2	0.09
17		102		2325	2328	3	0.13
18		103		2332	2333	1	0.04
19		104		2340	2337	-3	-0.13
20		105		2338	2337	-1	-0.04
Average for marble tile				2329	2329	0.4	0.09

The tile breaking strengths were tested and presented in Table 2.

Table 5. Average ceramic and marble tile weight changes before and after test.

	Weight before the test (g)	Weight after the test (g)	Difference (g)	Percent change (%)
Average for ceramic tiles	3847.6	3877.45	29.85	0.78
Standard deviation for ceramic tiles	34	34.7	7.4	0.19
Average for marble tiles	2329	2329.4	0.4	0.02
Standard deviation for marble tile	12.1	10.43	2.4	0.1

Breaking strengths of ceramic and marble tiles after treatment.

The breaking strength of the treated and the control tiles are in Table 6. Table 6 does not indicated the durations of test affects braking strength. The average breaking strength for ceramic tiles decreased from 431 to 408 lbs. The average breaking strength for marble tiles decreased from 690 to 609 lbs respectively (Tables 7). It seems that marble tiles are stronger than ceramic tiles. There is an average decrease in breaking strength 5% for ceramic tiles and 12% for marble tiles.

Table 6. The breaking strength of the treated and the control tiles.

Test Number	Tile	Tile Number	Treating Pressure (mmHg)	Total treating duration (min)	Breaking strength (lb)
1		1	250	46	415

2	Ceramic Tile	2		52	375
3		3		48	391
4		4		46	425
5		5		45	406
6		6		45	418
7		7		49	428
8		8		48	401
9		9		51	450
10		10		49	421
11		11	500	240	405
12		12		318	429
13		13	100	60	408
14		14		61	410
15		15		50	418
16	Marble Tile	101		55	386
17		102		53	376
18		103		53	395
19		104		55	384
20		105		54	426
Control	Ceramic Tile	31	Not treated		544
		32			610
		33			629
		34			573
	Marble Tile	106			689
		107			695
		108			716
		109			714

Table 7. Summary of T-test for comparison of breaking strength (lbs) of control and treated ceramic and marble tiles.

	Ceramic Tile			Marble tile		
	Treated	Control	Decrease	Treated	Control	Decrease
Mean	408.35	430.75	5%	609	689.5	12%
Variance	385.8	359.6		3080.5	1508.3	
Stand. Dev.	19.64	18.96		55.5	38.84	
n	20	4		5	4	
t	-2.0918	-2.4461				
degrees of freedom	22	7				
critical value	2.074	2.365				

These average observations of strengths before and after treatment are statistically significantly different. It is not known to what levels this change would affect installation and use of the tiles.

During the test, tiles were pressed and curves were recorded. Figure 55 shows the breaking strength and the deflection for a ceramic. More testing curves are shown in the Appendix.

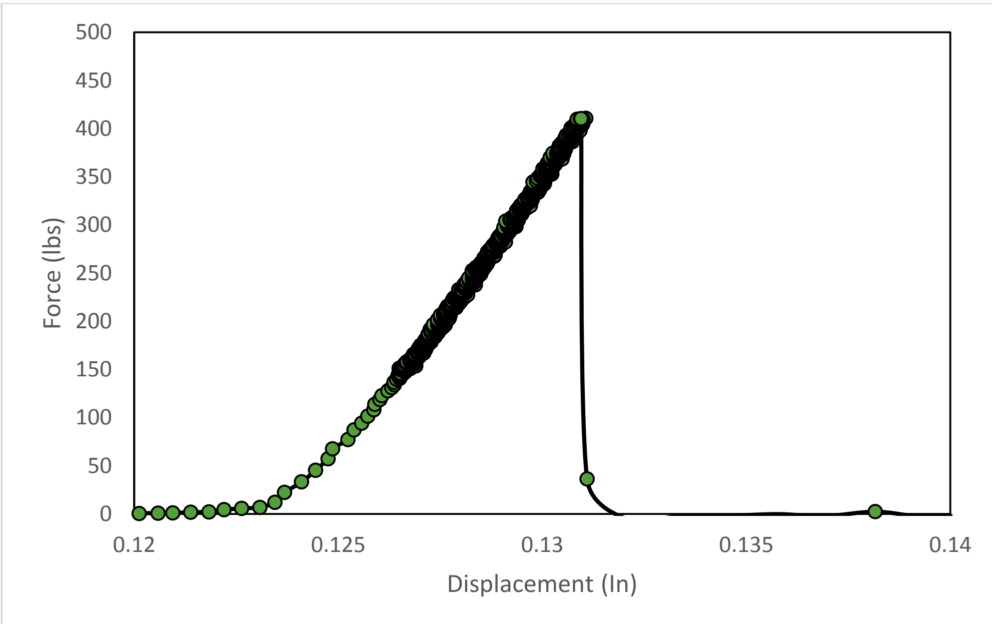


Figure 55. Breaking strength test from the treated ceramic tile # 1.

Warpage of ceramic tiles

Measure of warp for the treated and untreated tiles is in Table 8.

Table 8. Ceramic tile warpage of treated and untreated control samples

Tile	Warpage (×1000 Inches)							
	Side 1	Side 2	Side 3	Side 4	Diagonal 1	Diagonal 2	Warpage at side (%)	Warpage at diagonal (%)
Treated tiles	15	10	17	13	10	12	0.40	0.07
	5	13	3	4	-4	2	0.23	-0.01*
	20	10	15	9	9	9	0.49	0.06
	13	4	13	1	-1	-7	0.28	-0.03*
	18	13	6	12	5	11	0.45	0.05

	15	6	15	1	0	5	0.34	0.02
	19	11	15	13	12	9	0.53	0.07
	-1	16	4	15	-1	2	0.31	0.00
Average for treated tiles							0.38	0.03
Control tiles	3	15	6	15	0	0	0.35	0.00
	12	15	10	16	5	3	0.48	0.03
	13	16	11	20	8	8	0.55	0.05
	6	15	1	15	4	0	0.34	0.01
	8	15	14	15	8	7	0.47	0.05
	4	15	5	5	0	0	0.26	0.00
	10	15	12	17	8	5	0.49	0.04
	16	16	10	16	11	9	0.53	0.06
Average for control tiles							0.43	0.03

*(-) Represents the concave with respect to the face of the tile. Others are all convex to the surface of tiles.

The average warp of the edges of treated and untreated ceramic tiles were measured to be 0.38% and 0.43%. The average warp of the diagonals of treated and untreated tiles were the same at 0.03 %. T-test indicated there is no significant change during the treatment.

Effect of steam vacuum treatment on packaging

Figure 56 contains the result of ECT and Burst strength of corrugated paper board. Tests #1 to #4 are at initial pressure of 250 mmHg and test #5 at the initial pressure of 500 mmHg. All ECT and Burst test results were from corrugated paper boards after conditioning as required by the test protocol. However, the moisture content of the corrugated paper board after steaming is very high from 20-25%. Therefore, the corrugated paper boards after treatment and before conditioning would be very week. After corrugated dries out much of the strength is restored. The hot melt adhesive joints are unaffected by the treatment. The test also confirms that the longer the treatment cycle, the higher will be the MC of the corrugated. Higher initial vacuum levels reduce treatment time. The moisture content chance of the corrugated during treatment may be controlled by cycling vacuum and/or maintain a low pressure through out the treatment. Perhaps a final vacuum cycle can be used after treatment to reduce the moisture content of the corrugated before the packaging is used.

The unit load of tiles are shrink wrapped. The shrink wrap prevents liquid condensate from contacting the corrugated paper board packaging. The commercial operation may include a condensate traps above the treated units to prevent water from collecting on the surface of unit loads. The shrink film seems to be unaffected by the steam treatment. The quality of printing and integrity of gunned labels seem unaffected by the steaming. It is clear from these moisture content measurement of the corrugated immediately after treatment that handling of the packaging should be wait until the corrugated dries.

The T-tests were performed to compare the effect of vacuum steam treatment and the results were presented at Tables 9 and 10. There are no significant difference existed between the control samples and the treated samples after conditioning.

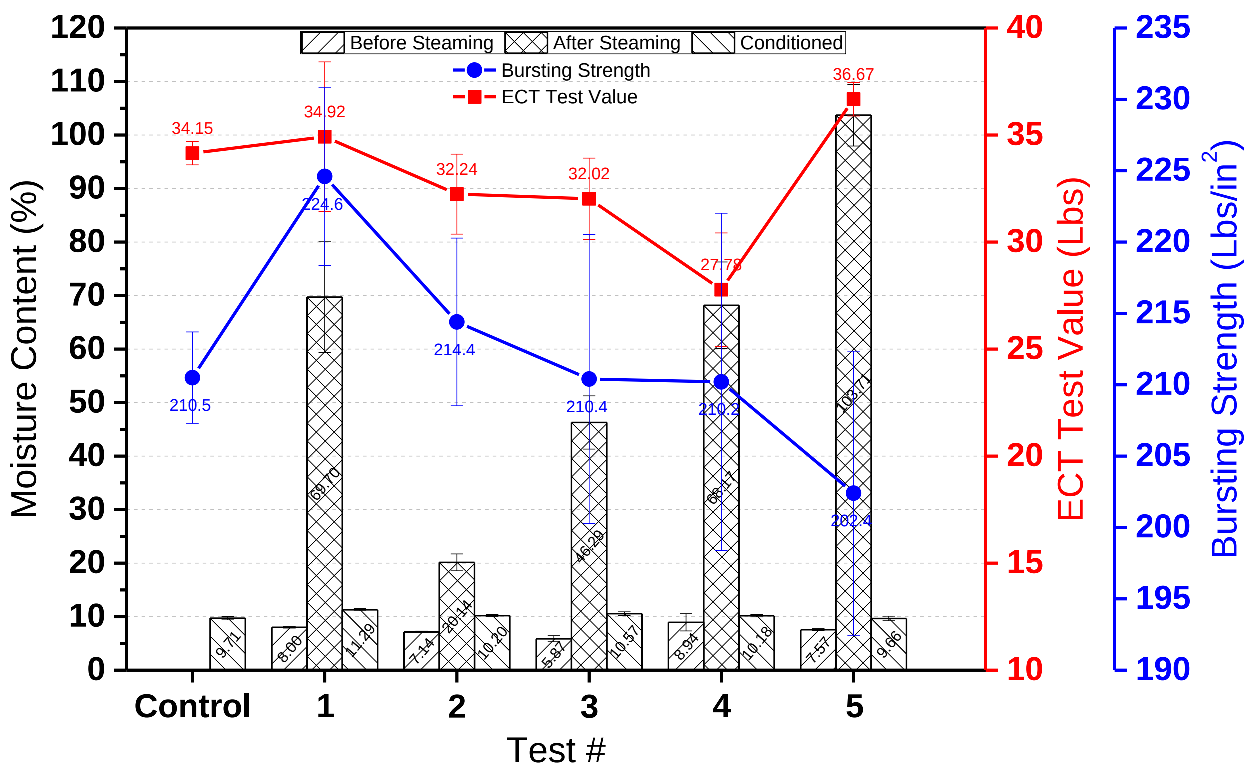


Figure 56. Properties of the control and treated corrugated paper boards.

Table 9. Statistical comparison of the average Burst strengths of control and treated paper boards.

	Control	Treated
Mean (lb/in^2)	210.5	214.4
Variance	113.6	525.1
Observations	10	30

Pooled Variance	427.6	
Hypothesized Mean Difference	0	
Df	38	
t Stat	-0.521	
P(T<=t) two-tail	0.605	
t Critical two-tail	2.02	

Table 10. Statistical comparison of the average ECT of control and treated paper boards.

	Control	Treated
Mean (lb)	34.15	32.46
Variance	3.27	75.79
Observations	10	29
Pooled Variance	58.15	
Hypothesized Mean Difference	0	
Df	37	
t Stat	0.61	
P(T<=t) two-tail	0.549	
t Critical two-tail	2.06	

Effect of vacuum steam treatment on the corrugated paper boxes

Moisture adsorption of the corrugated paper boxes

Paper board boxes were weighed before and after treatment and the results were presented in Table 11.

Table 11. The weight change of corrugated paper board boxes during treatment.

Test number	Treating pressure (mmHg)	Corrugated box	Weight before the test (g)	Weight after the test (g)	Difference (g)	Percent change (%)
13	100	1	169.7	175.7	6	3.54
		2	169.1	176.7	7.6	4.49
		3	169.1	184.9	15.8	9.34
		4	168.2	175.6	7.4	4.40
		5	167.4	176.2	8.8	5.26
		6	168.4	176.9	8.5	5.05
		7	167.9	173.3	5.4	3.22
		8	169.8	175.5	5.7	3.36

		9	168.7	174.7	6	3.56
		10	169.2	175.8	6.6	3.90
		11	168.1	174.1	6	3.57
		12	167.5	173.5	6	3.58
14		13	168.5	178.2	9.7	5.76
		14	170.0	176.3	6.3	3.71
		15	168.4	179.1	10.7	6.35
		16	169	180	11	6.51
		17	167.8	174.3	6.5	3.87
		18	166.5	175.6	9.1	5.47
		19	165.5	175	9.5	5.74
		20	165	175.6	10.6	6.42
		21	167.5	180.4	12.9	7.70
		22	168.1	179.7	11.6	6.90
		23	165.3	173.4	8.1	4.90
		24	171.6	180.1	8.5	4.95
15		25	167.7	172.2	4.5	2.68
		26	168.6	172.3	3.7	2.19
		27	168.4	174.6	6.2	3.68
		28	166.5	174	7.5	4.50
		29	169	175	6	3.55
		30	166.5	174.3	7.8	4.68
		31	167.5	171.4	3.9	2.33
		32	168.9	172	3.1	1.84
		33	167.9	173.7	5.8	3.45
		34	165.7	172.1	6.4	3.86
		35	166.3	171	4.7	2.83
		36	165	170.1	5.1	3.09
Average			167.9	175.4	7.47	4.45

The corrugated paper boxes picked up some moisture during the treatment. Moisture adsorption is a function of various characteristics of paper or board such as sizing, porosity, etc. From Table 11, average weights of paper boxes before and after treatment are 167.9 and 175.4 g respectively. The average box picked up 7.47 g of moisture or about a 4.45% increase in the weight.

According to the T-test in Table 12, there is a significant difference in the moisture content between the control sample and treated sample. Treated samples contained more moisture than control samples. Apparently, during the treatment, boxes picked up trace amount of moisture.

Table 12. Statistical comparison of the average moisture contents of control and treated paper boxes

MC		
t-Test: Two-Sample Assuming Equal Variances		
	Control	Treated
Mean (%)	8.66	8.89
Variance	0.141	0.110
Observations	36	108
Pooled Variance	0.118	
Hypothesized Mean Difference	0	
df	142	
t Stat	-3.5569	
P(T<=t) two-tail	0.00051	
t Critical two-tail	1.97	

Burst strength of the corrugated in the paperboard boxes

Average Burst strength of treated paperboard boxes is slightly higher than control boxes after the boxes are conditioned as shown in Figure 13. This difference is statistically significant.

Table 13. Statistical comparison of the Burst strengths of control and treated samples

t-Test: Two-Sample Assuming Equal Variances		
	Control	Treated
Mean (lb/in^2)	203.2	267.2
Variance	159.1	1454.1
Observations	21	69
Pooled Variance	1159.7	
Hypothesized Mean Difference	0	
df	88	
t Stat	-7.53116	
P(T<=t) two-tail	4.16E-11	
t Critical two-tail	1.98729	

Of the many criteria for boxes, compression strength is generally considered to be a prominent indicator of box performance. The compression strength is directly related to warehouse stacking performance. The laboratory test of box compression strength is easily performed and the result is useful for evaluation of the overall quality of the paper board materials (Maltenfort 1988). The statistical comparisons of the average ECT and average box compression of control and treated boxes are presented in Tables 14 and 15. There is no significant difference between control and treated boxes.

The moisture content, burst strength and ECT strengths of paper board boxes and other test results were presented in the Appendix.

Table 14. Statistical comparison of the ECT strengths of control and treated corrugated paperboard boxes.

t-Test: Two-Sample Assuming Equal Variances		
	Control	Treated
Mean (lb)	37.3	37.9
Variance	23.8	10.8
Observations	35	108
Pooled Variance	13.9	
Hypothesized Mean Difference	0	
df	141	
t Stat	-0.77748	
P(T<=t) two-tail	0.438176	
t Critical two-tail	1.976931	

Table 15. Statistical comparison of the compression strengths of control and treated corrugated paperboard boxes.

t-Test: Two-Sample Assuming Equal Variances		
	Control	Treated
Mean (lb)	406.9833	374.7353
Variance	12790.65	6072.311
Observations	6	17
Pooled Variance	7671.915	
Hypothesized Mean Difference	0	
df	21	

t Stat	0.775332	
P(T<=t) two-tail	0.446787	
t Critical two-tail	2.079614	

7. Conclusion

1. 56°C for 30 minutes using vacuum and steam is effective for killing Mediterranean snails, *Eobania vermiculata* and *Cernuella cisalpine*, within packaged unit loads of tile. Initial vacuum level significantly affect treatment duration. Using 100 mmHg and 250 mmHg initial vacuum, the average cycle time to reach 56°C/30 within all regions of the unit load was less than 61 minutes with average total treating time of 51.1 minutes. At 500 mmHg initial vacuum level, the average cycle time increase to more than 183 minutes.

2. The steam and vacuum treatment reduced the overall breaking strength of the ceramic tile by 5% and the marble tile by 12%. It is not known whether this would affect the use of these tiles.

3. The steam and vacuum treatment initially reduces the strength of the corrugated paper board packaging. However, as the corrugated dries, the properties are recovered. This indicates that a drying cycle at the end of the treatment should be used before the packaging is used.

4. The snails will lose weight during treatment. The weight of *Cernuella cisalpina* ranges about 0.096 g. It was found that *Eobania vermiculata* lost an average of 18.9% body weight during the treatment.

5. Vacuum steam treatment does not cause the tile to warp.

6. The color of tiles is not affected by the treatment. The ceramic tiles adsorbed about 0.78% moisture during treatment. The marble tiles absorb no moisture.

7. Quality of packaging, printing and hot melt glued connections is unaffected by the vacuum steam treatment. Gunned labels remain intact and usually were unaffected by the treatment process.

8. The shrink film seems to be affected by the treatment. However condensations of moisture during treatment could be controlled to prevent contact of the packaging by liquid water.

8. References

Baker G. H., 2008. “The population dynamics of the Mediterranean snails Cernuella virgata, Cochlicella acuta (Hygromiidae) and Theba pisana (Helicidae) in pasture-cereal rotations in South Australia: a 20-year study,” Australian Journal of Experimental Agriculture, vol. 48, no. 12, pp. 1514–1522.

Baker G. H., S. Beckett, and B.Thammavongsa. 2012. “Are the European snails, Theba pisana (M¨uller, 1774) (Helicidae), Cernuella virgata (da Costa, 1778) and Cochlicella acuta (M¨uller, 1774) (Hygromiidae) attracted by potential food odours?” Crop Protection, vol. 42, no. 3, pp. 88–93.

Bean D. 2013. http://www.mdinvasivesp.org/archived_invaders/archived_invaders_2013_03.html

Chen, Z., M.S. White and Y. Wu. 2012. Vacuum steam phytosanitation of hardwood pallets and pallet stringers. Forest Products Journal. 62(5):378-382.

Civeyrel, L., and D. Simberloff. 1996. A tale of two snails: is the cure worse than the disease? Biodiversity and Conservation 5: 1231–1252.

Cotton, R. T. (1963). Pests of stored grain and grain products. Burgess Publishing Co., Minneapolis, Minnesota, 318 pp.

Cowie R. H., R. T. Dillon Jr., D. G. Robinson and J. W. Smith. 2009. “Alien non-marine snails and slugs of priority quarantine importance in the United States: a preliminary risk assessment,” The American Malacological Bulletin, vol. 27, no. 1-2, pp. 113–132.

Fagbuaro O., J.A. Oso and J.B. Edward. 2006. Nutritional status of four species of giant land snails in Nigeria. Journal of Zhejiang Univ Sci B. Sep; 7(9): 686–689.

Flint, M. L. 1998. Pests of the Garden and Small Farm: A Grower’s Guide to Using Less Pesticide. 2nd ed. Oakland: Univ. Calif. Agric. Nat. Res. Publ. 3332.

Godan, D. 1983. Pest Slugs and Snails: Biology and Control. Springer-Verlag, Berlin, Germany. 445 pp.

Hansen, J. D. (2001). Ultrasound treatments to control surface pests of fruit. Horticulture Technology. 11: 186-188

Hollingsworth, R.G., Kenata, R. Sullivan, J.J. Bishop, H.S. Qvarnstrom, Y. DaSilva and D.G. Robinson, 2007. Distribution of Parmarion cf. martensi (Pulmo-nata: Helicarionidae), a new semi-slug pest on Hawai’i Island, and its potential as a vector for human angiostrongyliasis. Pacific Science 61(4): 457–467

Lydeard, C., R.H. Cowie, W.F. Ponder, A.E. Bogan, P. Bouchet, S.A. Clarke, K.S. Cummings, T.J. Frest, O. Gargominy, D.G. Herbert, R. Hershler, K.E. Pe-Rez, B. Roth, M. Seddon, E.E. Strong and F.G. Thompson. 2004. The global decline of non-marine mollusks. Bioscience 54(4): 321–330.

Maltenfort, G. G. (1988). Compression load distribution on corrugated boxes. Paperboard Packaging. Sep. pp110.

Pimentel, D., R. Zuniga and D. Morrison. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecological Economics 52 (2005) 273–288.

Prusky, D., Y. Fuchs, I. Kobiler, I. Roth, A. Weksler. Y. Shalom. E. Fallik. M. Scholler. G. Zauberman and E. Pesis. 1999. Effect of hot water brushing, prochloraz treatment and waxing on the incidence of black spot decay caused by Alternaria alternata in mango fruits. Schroeder, Postharvest Biology and Technology 15, 165-174.

Solem, A., 1984. A world model of land snail diversity and abundance. In: Solem A., van Bruggen A.C. (Eds.), World-Wide Snails: Biogeographical Studies on Non-Marine Mollusca. Leiden, pp. 6–22.

Steinke D., C. Albrecht and M. Pfenninger. 2004. Molecular phylogeny and character evolution in the Western Palearctic Helicidae s.l. Gastropoda: Stylommatophora) Mol. Phylogenet. Evol., 32 (2004) 724–734.

Tang J. 2007. Heat Treatments for Postharvest Pest Control. Cromwell Press. Trowbridge. UK. pp350.

Texas Invasive Species Institute. n.d. Retrieved from http://www.tsusinvasives.org/database/choco-band-snail.html.

USDA. 2005. USDA-Animal and Plant Health Inspection Service-Plant Protection and Quarantine Treatment Manual: Tl06. Riverdale, Maryland, pp.5.2.69-5.2.75.

U.S. Habitat: In a broad variety of habitats, usually in dry vegetation, mainly in coastal vicinity, also in agricultural crops and vineyards.

Ferguson I.B., S. Ben-Yehoshua, E.J. Mitcham, R.E. McDonald, S. Lurie. (2000). Postharvest heat treatments: introduction and workshop summary. Postharvest Biology and Technology 21 1–6.

Puizina J, Sanja Puljas, Celjana Fredotovi, Ivica Šamani, and Grgur Plesli. 2013, Phylogenetic Relationships among Populations of the Vineyard Snail Cernuella virgata (Da Costa, 1778), ISRN Zoology Volume 2013, Article ID 638325, 9 pages.

10. Appendix A

Table A1. The burst strength and ECT of corrugated paper board boxes.

Treatment	Box #	Face #	Average Moisture Content	Average Bursting Strength	Average ECT Strength
			(%)	(Lbs/in2)	(Lbs)
Control					
	Box 1		8.63	203	37.55
		Face 1	8.77	195	37.93
		Face 2	8.15		34.33
		Face 3	8.48	210	37.6
		Face 4	8.9		34.74
		Face 5	8.27	210	42.6
		Face 6	10		49.8
	Box 2		8.44	198	34.75
		Face 1	8.38	187	35.68
		Face 2	8.58		38.07
		Face 3	8.08	199	36.62
		Face 4	8.97		37.93
		Face 5	8.87	215	24.4
		Face 6	7.98	200	21.75
	Box 3		8.81	209	37.32
		Face 1	8.97	205	37.33
		Face 2	9.16		36.63
		Face 3	8.62	202	37.7
		Face 4	8.89		36.68
		Face 5	8.73	218	38.45
		Face 6	8.54	230	36.7
	Box 4		8.77	200	36.82
		Face 1	8.93	189	35.7
		Face 2	8.61		39.56

		Face 3	8.86	210	37.94
		Face 4	8.85		39.18
		Face 5	8.66		27.7
		Face 6	8.19	200	38.7
	Box 5		8.65	196	38.93
		Face 1	8.51	185	38.92
		Face 2	8.89		38.72
		Face 3	8.68	198	37.46
		Face 4	8.71		39.46
		Face 5	8.57		39.05
		Face 6	8.44	225	44.5
	Box 6		8.67	196	39.06
		Face 1	8.8	185	38.8
		Face 2	8.65		40.38
		Face 3	8.83	205	40.66
		Face 4	8.26		37.28
		Face 5	8.82	190	37.17
		Face 6	8	210	
Vacuum/Steam					
	Box 1		8.72	255	38.46
		Face 1	8.75	247	38.6
		Face 2	8.23		38.68
		Face 3	8.4	243	39.57
		Face 4	9.47		38.3
		Face 5	8.9	310	37.3
		Face 6	9	260	37.55
	Box 2		8.95	198	37.83
		Face 1	8.8	187	38.38
		Face 2	8.62		38.52
		Face 3	9.13	205	35.74
		Face 4	9.15		37.34
		Face 5	8.97	185	37.34
		Face 6	9.06	220	41.15
	Box 3		9.04	251	37.64
		Face 1	8.88	200	39.07
		Face 2	9.19		38.74
		Face 3	8.8	265	38.33
		Face 4	9.27		41.03
		Face 5	9.44	280	44.25
		Face 6	8.91	280	20.05
	Box 7		8.62	283	39.26

		Face 1	8.71	257	40.3
		Face 2	8.57		42.8
		Face 3	8.48	287	35.87
		Face 4	8.6		42.2
		Face 5	8.65	340	31.8
		Face 6	8.73	295	40.55
	Box 8		8.72	197	37.47
		Face 1	8.62	195	40.58
		Face 2	8.72		37.04
		Face 3	8.7	203	36.9
		Face 4	8.92		38.74
		Face 5	8.61	220	37.7
		Face 6	8.87	170	28.75
	Box 9		8.83	281	38.92
		Face 1	8.5	265	36.2
		Face 2	8.85		35.87
		Face 3	9.01	292	37.67
		Face 4	8.96		39.73
		Face 5	8.8	295	45.55
		Face 6	9.02	285	41.6
	Box 13		8.94	281	36.93
		Face 1	9.01	273	35.13
		Face 2	9.02		39.03
		Face 3	8.96	283	39.8
		Face 4	8.88		32.4
		Face 5	8.97	270	41.8
		Face 6	8.71	300	35
	Box 14		9.08	288	36.89
		Face 1	9.07	292	35.1
		Face 2	8.89		39.33
		Face 3	9.12	292	39.93
		Face 4	9.01		36.97
		Face 5	9.24	310	31.75
		Face 6	9.11	245	36.4
	Box 15		9.1	267	39.11
		Face 1	9.41	230	39.87
		Face 2	8.51		38.3
		Face 3	9.3	273	39.4
		Face 4	8.58		36.9
		Face 5	9.04	305	42.7
		Face 6	9.27	320	38.45

	Box 19		8.89	268	37.87
		Face 1	9.06	283	40.63
		Face 2	9.33		38.1
		Face 3	8.52	267	40.07
		Face 4	8.8		37.17
		Face 5	7.65	230	28.75
		Face 6	10.15	260	40.25
	Box 20		8.93	301	39.26
		Face 1	8.71	317	36.6
		Face 2	9.12		37.93
		Face 3	8.73	290	39.33
		Face 4	8.82		36.77
		Face 5	9.01		45.15
		Face 6	9.6	285	42.95
	Box 21		8.91	293	37.83
		Face 1	8.71	295	37.93
		Face 2	9.69		40.37
		Face 3	9.26	307	34.93
		Face 4	8.56		37.5
		Face 5	8.54	240	38.25
		Face 6	8.42	300	38.7
	Box 25		8.74	271	38.39
		Face 1	8.77	288	35.9
		Face 2	8.85		39.93
		Face 3	8.68	282	38.87
		Face 4	8.65		36.97
		Face 5	8.88	185	37.2
		Face 6	8.63		42.4
	Box 26		9.06	278	37.65
		Face 1	9.14	290	35.37
		Face 2	9.03		39.83
		Face 3	8.93	267	35.97
		Face 4	8.78		35.67
		Face 5	9.47	300	42.1
		Face 6	9.04	255	38.85
	Box 27		9.09	278	36.4
		Face 1	9.16	278	35.07
		Face 2	9.01		35.4
		Face 3	9.26	260	35
		Face 4	9.11		39.23
		Face 5	8.77	310	37.75

		Face 6	8.71	300	37.75
	Box 31		8.79	251	36.63
		Face 1	8.84	227	35.97
		Face 2	9.39		37.23
		Face 3	8.4	253	36.77
		Face 4	9.07		35.7
		Face 5	8.66	280	37.6
		Face 6	8.65	290	37.45
	Box 32		8.72	292	37.81
		Face 1	8.57	285	37.93
		Face 2	8.74		37
		Face 3	9.01	305	37.4
		Face 4	8.58		35.7
		Face 5	8.76	315	38.45
		Face 6	8.56	250	42
	Box 33		8.86	241	37.12
		Face 1	8.65	223	36.67
		Face 2	9.2		37.23
		Face 3	9.11	240	36.4
		Face 4	9.02		36.7
		Face 5	8.45	300	40.3
		Face 6	8.54		36.15

Table A2. Corrugated paper box compression strength.

			Maximum Load	Defection
	Test #	Box #	(lbs)	(in)
Control Sample		1	432.5	0.211
		2	331.4	0.388
		3	355.5	0.171
		4	622.5	0.458
		5	381.6	0.436
		6	318.4	0.152
Vacuum/Steam		4	402.6	0.323
		5	421.8	0.510
		6	419.8	0.384
		10	409.3	0.414
		11	504.0	0.408
		12	389.9	0.179

		16	340.1	0.212
		17		
		18	294.7	0.324
		22	257.1	0.290
		23	227.2	0.255
		24	252.0	0.313
		28	346.0	0.154
		29	446.5	0.316
		30	443.7	0.334
		34	367.0	0.328
		35	425.3	0.205
		36	423.5	0.314

Table A3. The Burst and ETC strengths of corrugated paper board.

Sample #	Condition	ECT Test				Burst Test			
		Moisture Content (%)			ECT Test (Lbs)	Moisture Content (%)			Burst Test (Lbs/in^2)
		Before Steam	After Steam	Condi- tioned		Before Steam	After Steam	Conditioned	
0-1	Control			10.0	36.8			9.7	215
0-2				9.1	35.6			9.8	225
0-3				11.6	36.0			9.2	225
0-4				9.2	33.8			9.6	220
0-5				10.1	31.1			9.9	200
0-6				10.9	33.4			9.5	210
0-7				9.0	34.5			10.1	200
0-8				9.0	34.9			9.2	205
0-9				8.2	33.7			7.2	195
0-10				10.0	31.7			8.5	210
1-6	Treated at 250 mmHg	9.2	140.0	14.2	67.4	8.2	123.0	11.8	240
1-7		8.1	107.3	12.2	35.5	8.4	119.1	10.7	210
1-8		8.2	127.9	10.7	28.6	8.0	93.3	11.0	200
1-9		7.4	47.9	11.6	29.1	7.7	26.9	10.4	205
1-10		6.8	58.5	11.0	30.9	7.8	32.3	11.6	200
1-11		7.4	23.0	9.8	32.9	8.4	32.4	11.4	220
1-12		8.3	25.6	10.7	32.4	8.2	25.9	11.1	225
1-13		7.4	61.5	11.5	29.0	7.7	27.2	11.4	250

1-14		9.1	166.9	12.4	29.3	8.0	24.1	11.0	241
1-15		7.4	29.5	9.8	34.1	8.4	101.7	11.4	255
2-1		6.6	14.0	9.9	33.3	7.9	16.7	10.5	229
2-2		7.5	13.3	10.0	39.9	7.1	23.9	11.1	228
2-3		6.6	21.3	9.0	28.6	7.0	19.8	9.7	195
2-4		6.6	29.5	10.7	30.5	7.0	17.5	9.9	215
2-5		8.1	26.6	11.3	28.9	7.2	18.6	9.8	205
3-1		5.0	77.7	9.1	29.7	7.2	28.0	11.4	240
3-2		7.4	60.7	10.7	34.8	4.0	21.8	10.6	191
3-3		6.6	41.0	12.3	25.1	6.3	51.5	10.2	195
3-4		1.6	58.3	8.7	37.3	6.2	34.9	10.4	236
3-5		7.4	43.4	11.5	33.2	7.3	45.7	10.9	190
4-1	Treated at 500 mmHg	8.5	27.4	9.4	19.4	7.2	92.5	10.5	198
4-2		6.7	94.2	10.8	27.7	7.2	56.7	10.8	165
4-3		7.6	105.1	9.3		24.0	41.0	11.0	240
4-4		7.4	89.3	9.8	30.2	7.2	66.2	10.2	228
4-5		5.7	37.7	9.0	33.8	7.8	71.7	10.9	220
5-1		8.2	80.3	10.7	13.0	7.9	115.2	9.9	210
5-2		7.4	96.7	7.4	36.8	7.6	65.6	10.9	211
5-3		8.2	129.5	8.2	35.4	7.3	115.2	10.9	229
5-4		7.4	121.3	7.4	39.2	6.9	106.6	10.5	162
5-5		8.3	106.6	9.9	35.3	6.6	100.0	10.8	200

Table A4. *Eobania vermiculata* snail weight.

Test number	Treating pressure (mmHg)	*Eobania vermiculata*	Weight before the test (g)
2	250	1	5.15
		2	4
		3	1.5
		4	1.38
		5	4.69
		6	4.95
		7	4.43
		8	4.09
		9	3.89
		10	4.31
		11	2.77
		12	5.09

		13	3.24
		14	3.96
		15	5.02
		16	3.06
		17	0.59
		18	3.65
		19	5.71
		20	3.68
		21	3.75
		22	3.49
		23	3.58
		24	1.92
Average			

Table A5. Snail weight change during the test.

Test number	Treating pressure (mmHg)	*Eobania vermiculata*	Weight before the test (g)	Weight after the test (g)	Difference	Percentage change (%)
5	250	1	3.27	2.89	0.38	11.62
		2	0.52	0.42	0.1	19.23
		3	0.25	0.16	0.09	36.00
		4	4.76	4.37	0.39	8.19
		5	0.41	0.33	0.08	19.51
		6	0.1	0.09	0.01	10.00
		7	3.55	3.52	0.03	0.85
		8	0.59	0.47	0.12	20.34
		9	0.16	0.15	0.01	6.25
		10	5.82	4.82	1	17.18
		11	0.56	0.35	0.21	37.50
		12	0.18	0.18	0	0.00
		13	3.36	2.23	1.13	33.63
		14	1.82	1.48	0.34	18.68
		15	0.31	0.22	0.09	29.03
		16	3.18	2.93	0.25	7.86
		17	0.48	0.36	0.12	25.00
		18	0.28	0.23	0.05	17.86
		19	3.93	3.69	0.24	6.11
		20	1.46	1.36	0.1	6.85
		21	0.16	0.11	0.05	31.25
		22	4.38	3.63	0.75	17.12
		23	4.10	2.84	1.26	30.73
		24	0.79	0.45	0.34	43.04
Average			1.23	1.04	0.30	18.91

Table A6. Average weight change *Eobania vermiculata* during the tests.

Test Number	Treating Pressure (mmHg)	Chocolate Snail	Weight before the test (g)	Weight after the test (g)	Difference	Percentage change (%)
9	250	1	5.75	4.81	0.27	16.57
		2	3.33	3.1	0.28	16.45
		3	0.73	0.64	0.29	16.72
		4	5.14	4.12	0.30	17.38
		5	2.35	1.74	0.31	17.25
		6	0.99	0.63	0.32	17.71
		7	3.78	2.85	0.29	17.74
		8	3.14	2.63	0.29	16.91
		9	0.84	0.6	0.31	17.62
		10	5.52	5.1	0.27	16.95
		11	3.98	3.48	0.27	16.88
		12	0.15	0.19	0.28	16.37
		13	4.16	3.57	0.28	16.73
		14	3.39	3.1	0.29	16.38
		15	3.58	3.15	0.29	16.32
		16	4.39	4.12	0.30	16.74
		17	4.25	3.38	0.31	17.16
		18	0.41			
		19	4.22	3.53	0.28	15.86
		20	4.17	3.28	0.24	15.24
		21	0.99			
		22	4.32	3.44	0.23	13.29
		23	3.9	2.64	0.24	13.84
		24	0.72	0.54	0.25	14.42
Average			2.06	1.68	0.28	16.39

Table A7. Average weight of *Cernuella cisalpine* during the test.

Test Number	Treating Pressure (mmHg)	*Cernuella cisalpina*	Weight before the test (g)
3	250	1	0.11
		2	0.16
		3	0.14
		4	0.09
		5	0.14
		6	0.08

		7	0.09
		8	0.16
		9	0.11
		10	0.15
		11	0.09
		12	0.08
		13	0.07
		14	0.08
		15	0.06
		16	0.11
		17	0.08
		18	0.09
		19	0.14
		20	0.10
		21	0.05
		22	0.09
		23	0.12
		24	0.08
		25	0.07
		26	0.13
		27	0.04
		28	0.16
		29	0.11
		30	0.10
		31	0.15
		32	0.10
		33	0.13
		34	0.08
		35	0.06
		36	0.08
		37	0.07
		38	0.06
		39	0.09
		40	0.04
		41	0.05
		42	0.08
		43	0.08
		44	0.08
		45	0.08
Average			0.096

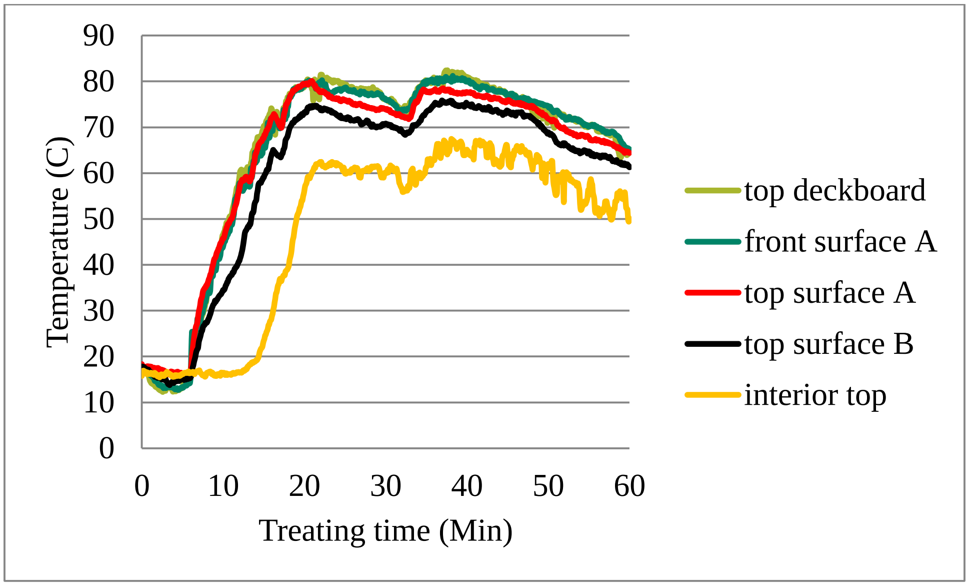


Figure A1. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 1.

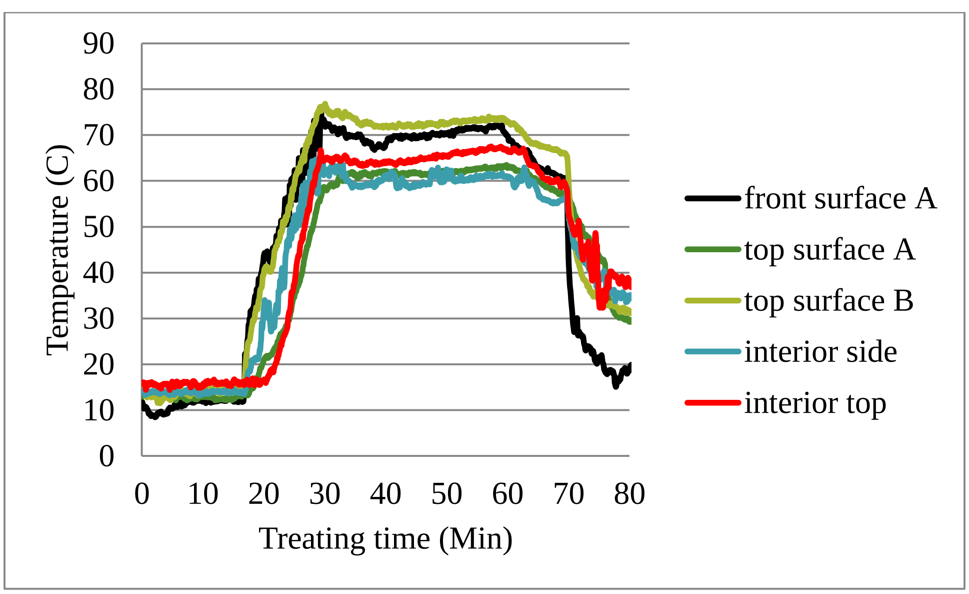


Figure A2. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 2.

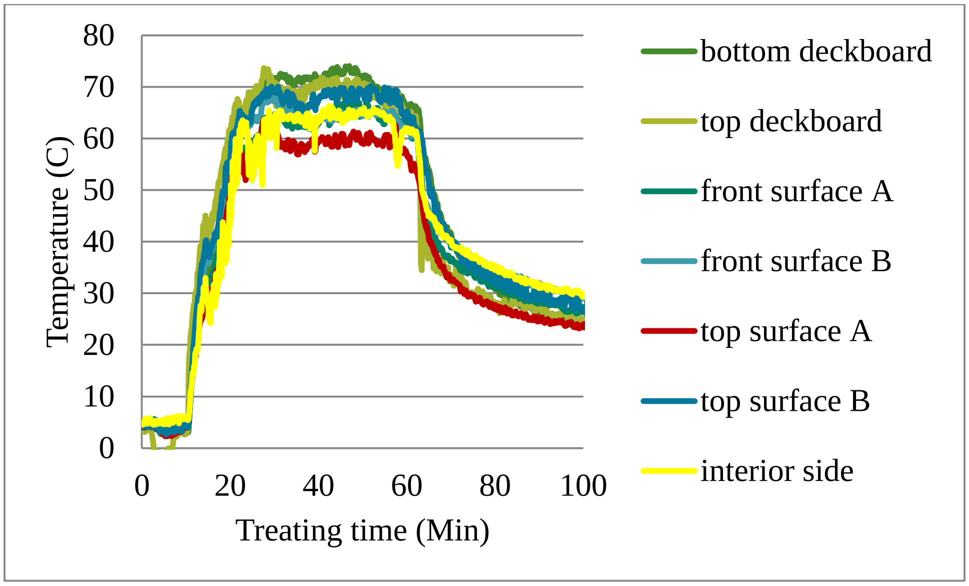


Figure A3. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at the test 3.

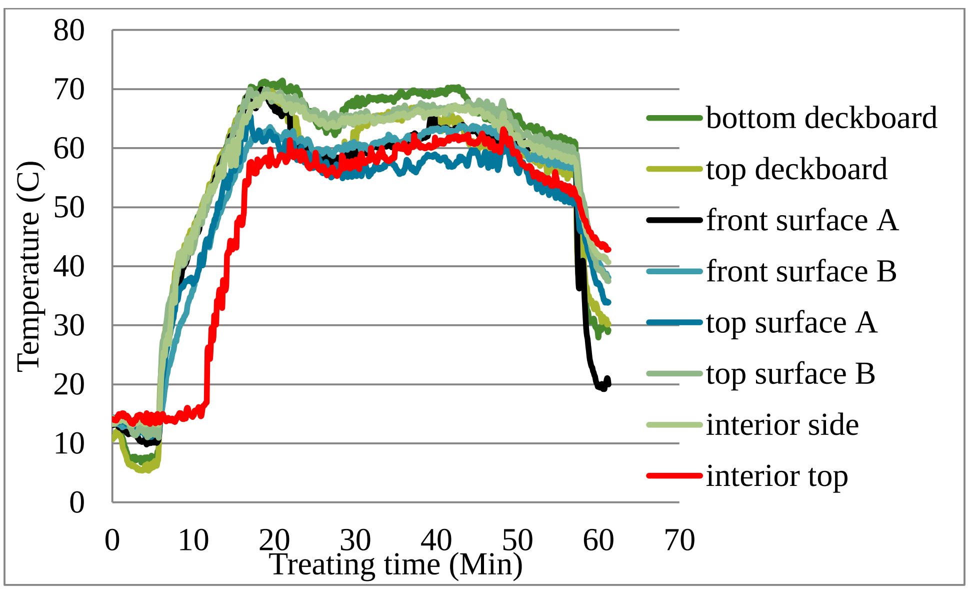


Figure A4. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 4.

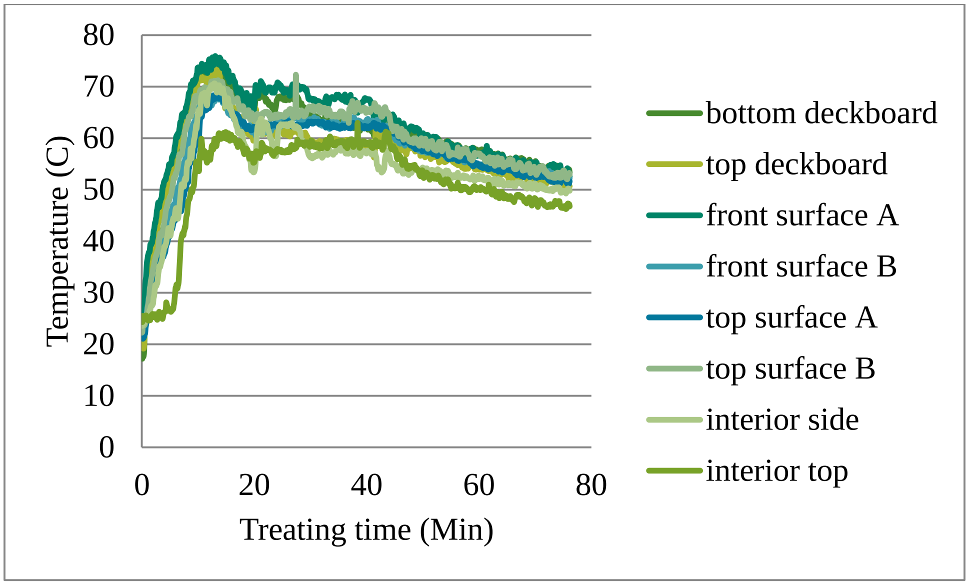


Figure A5. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 5.

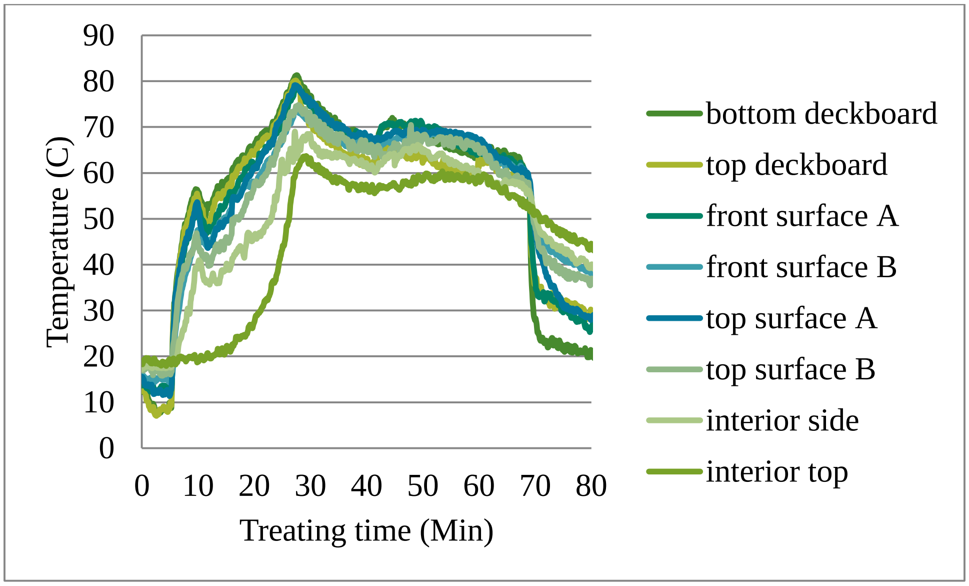


Figure A6. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 6.

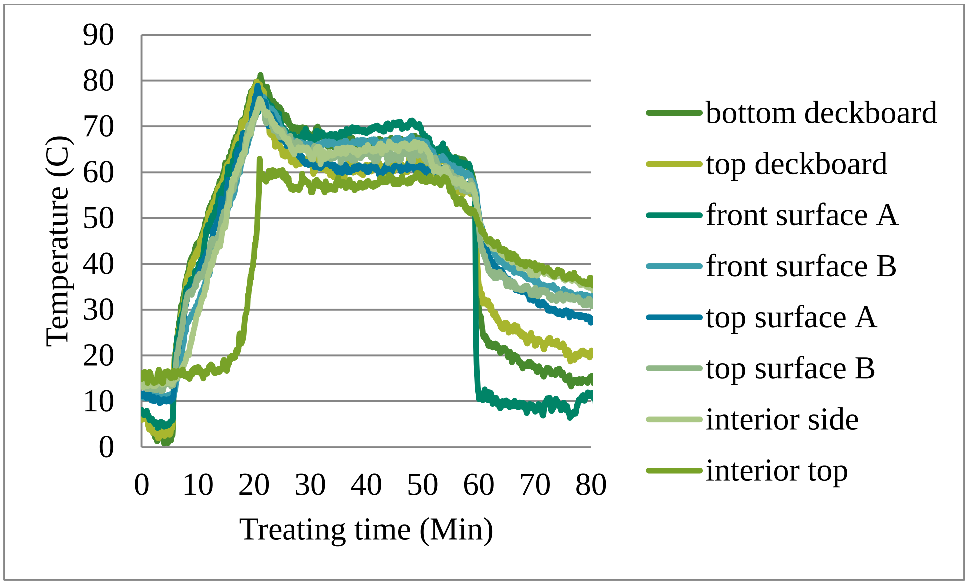


Figure A7. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 7.

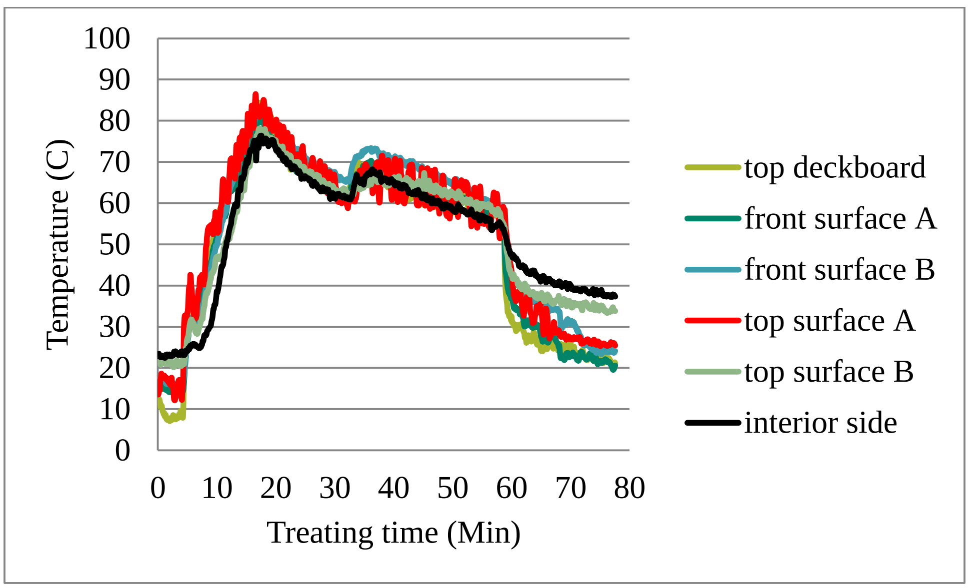


Figure A8. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 8.

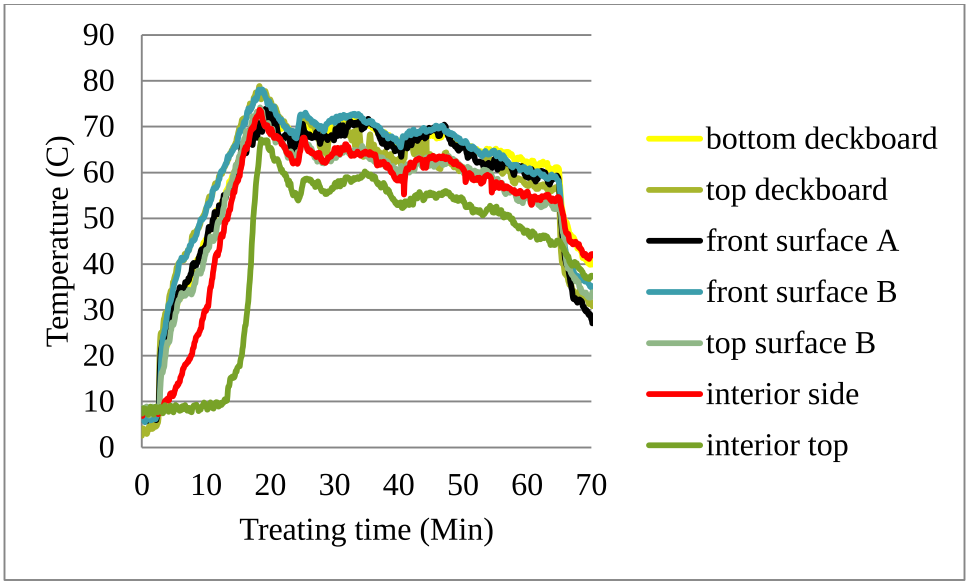


Figure A9. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 9.

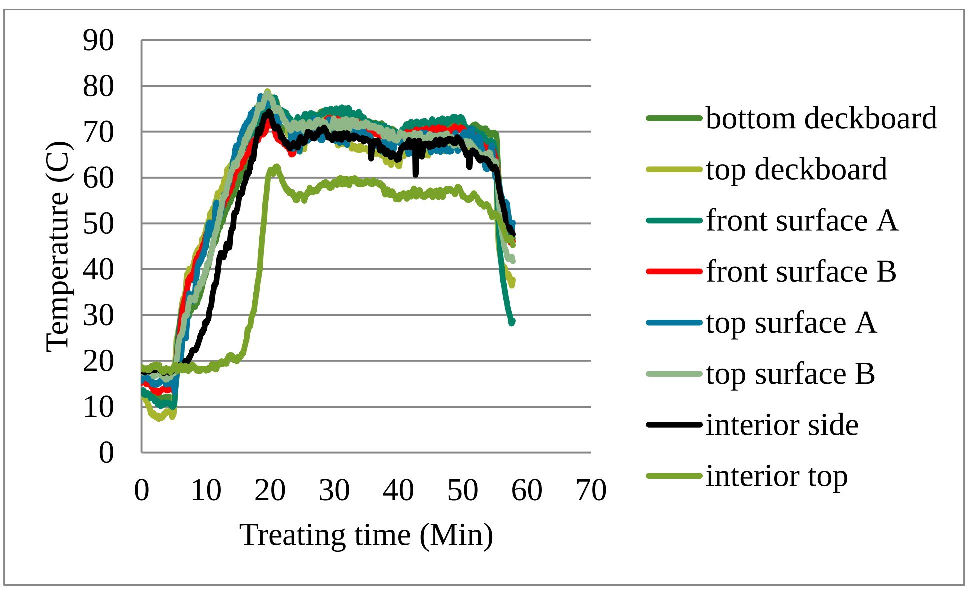


Figure A10. Temperature profile during treatment at the initial vacuum pressure of 250 mmHg at test 10.

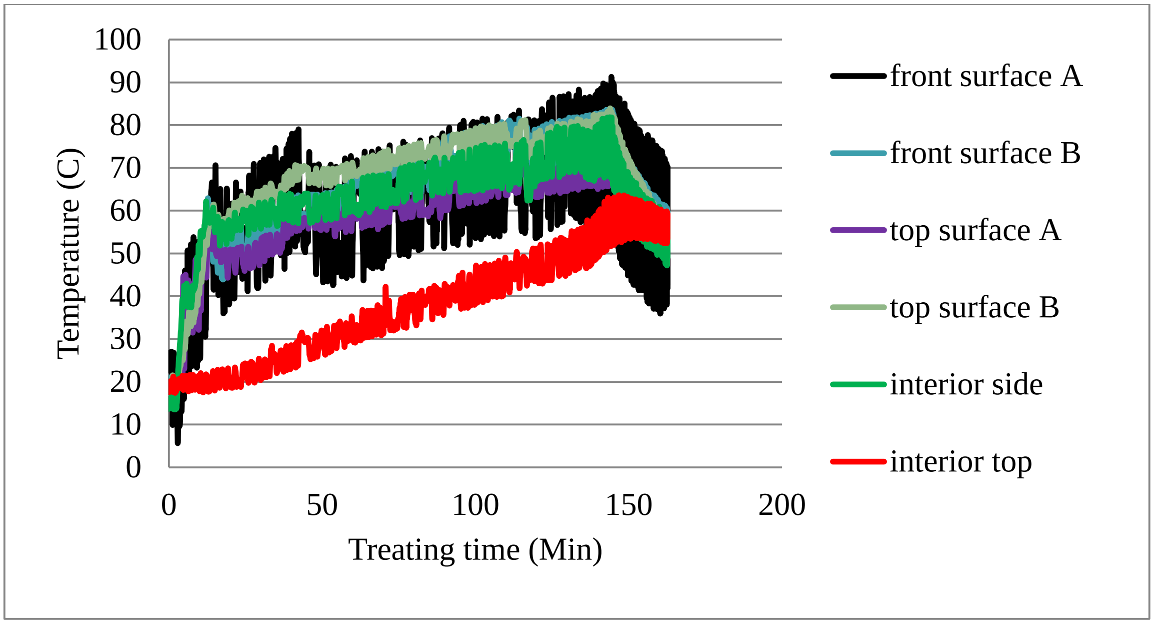


Figure 11. Typical temperature profile at the initial vacuum pressure of 500 mmHg at test 11.

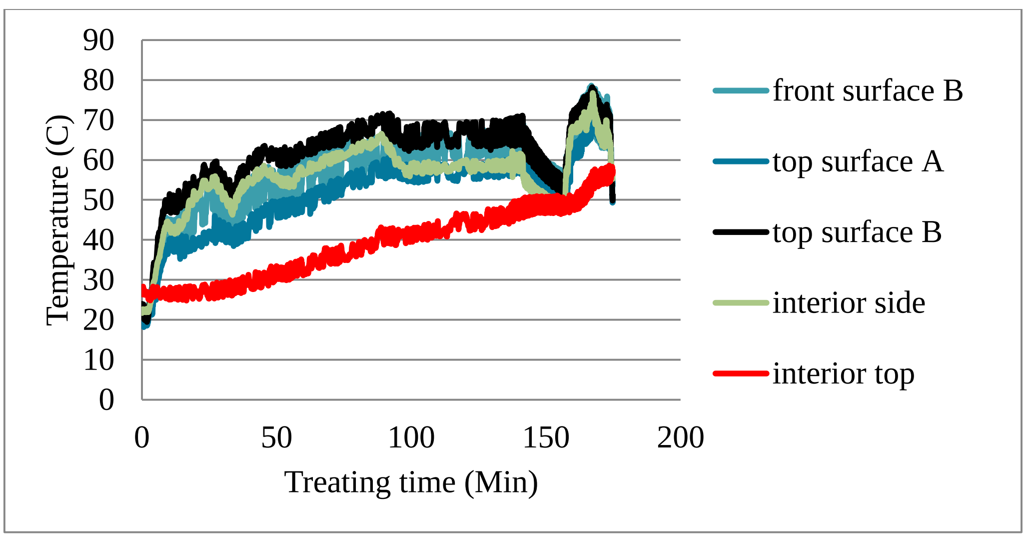


Figure 12. Temperature profile during treatment at the initial vacuum pressure of 500 mmHg at test 12.

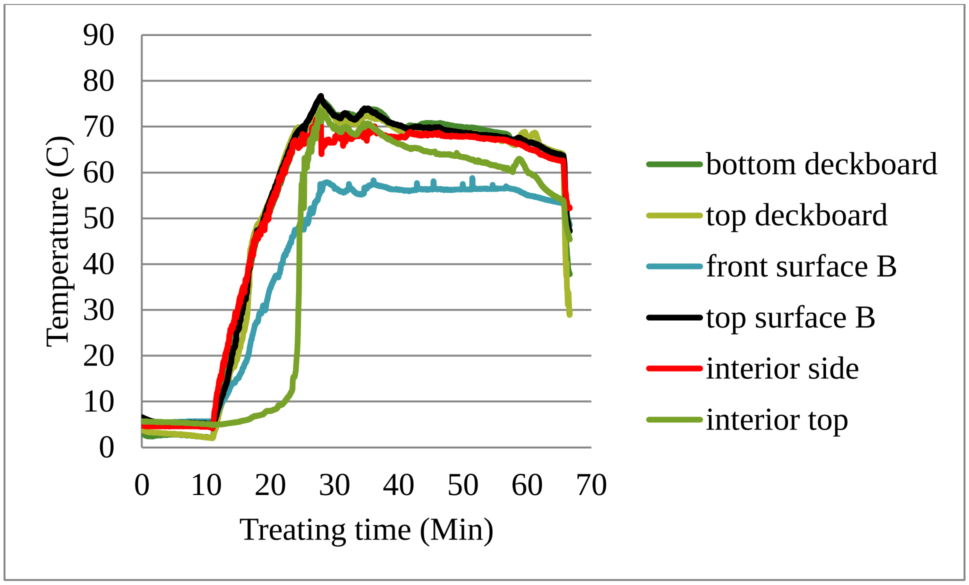


Figure 13. Temperature profile during treatment at the initial vacuum pressure of 100 mmHg at test 13.

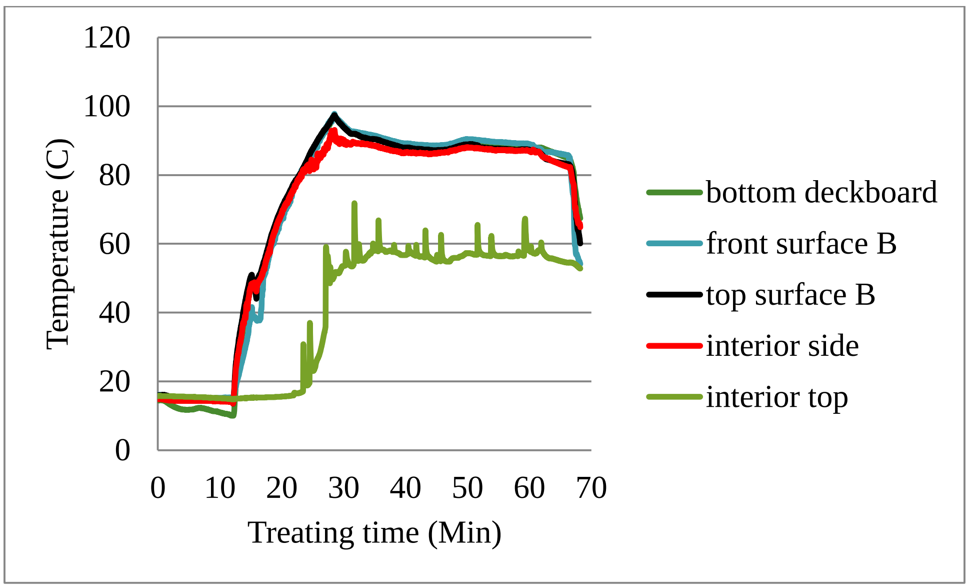


Figure A14. Temperature profile during treatment at the initial vacuum pressure of 100 mmHg at test 14.

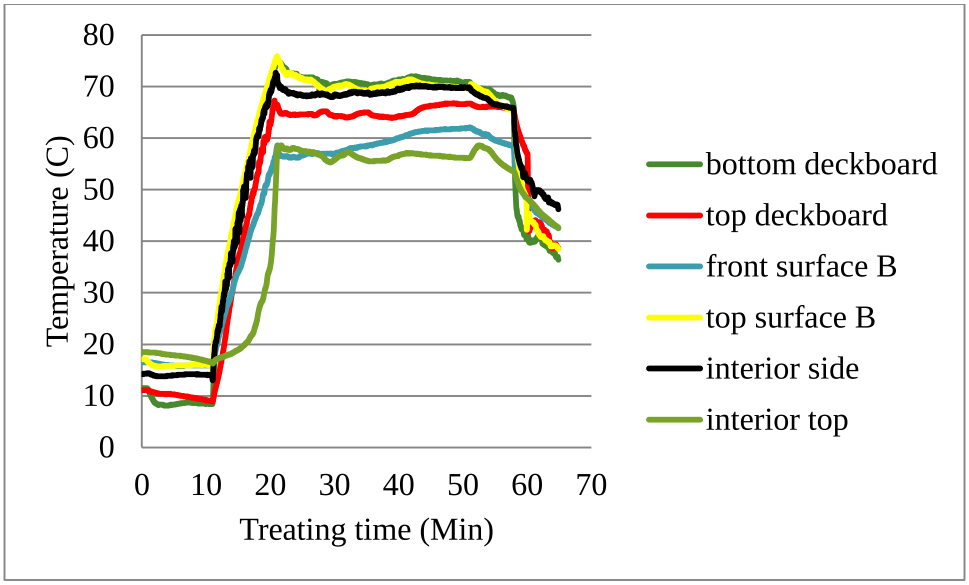


Figure A15. Temperature profile during treatment at the initial vacuum pressure of 100 mmHg at test 15

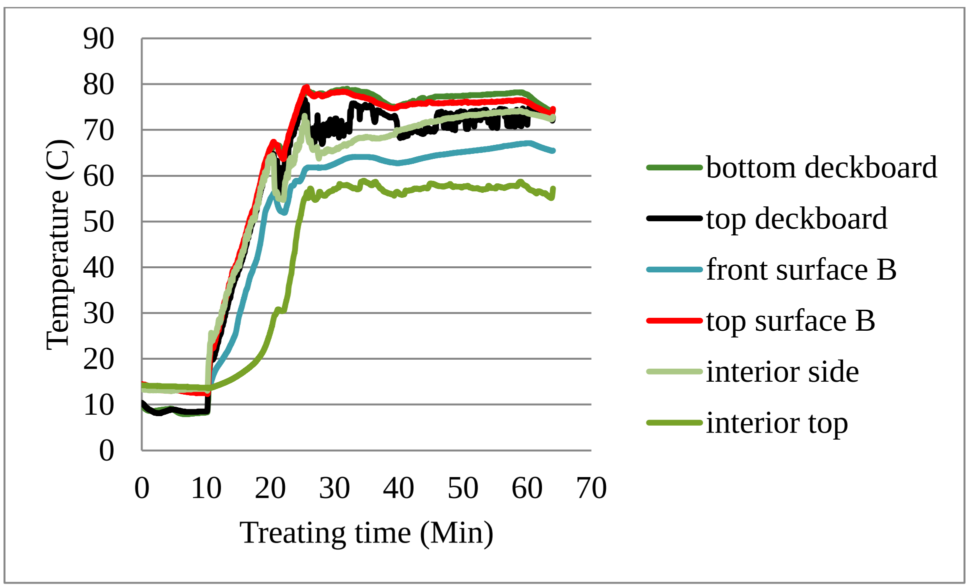


Figure 16. Temperature profile during treatment at the initial vacuum pressure of 100 mmHg at test 16.

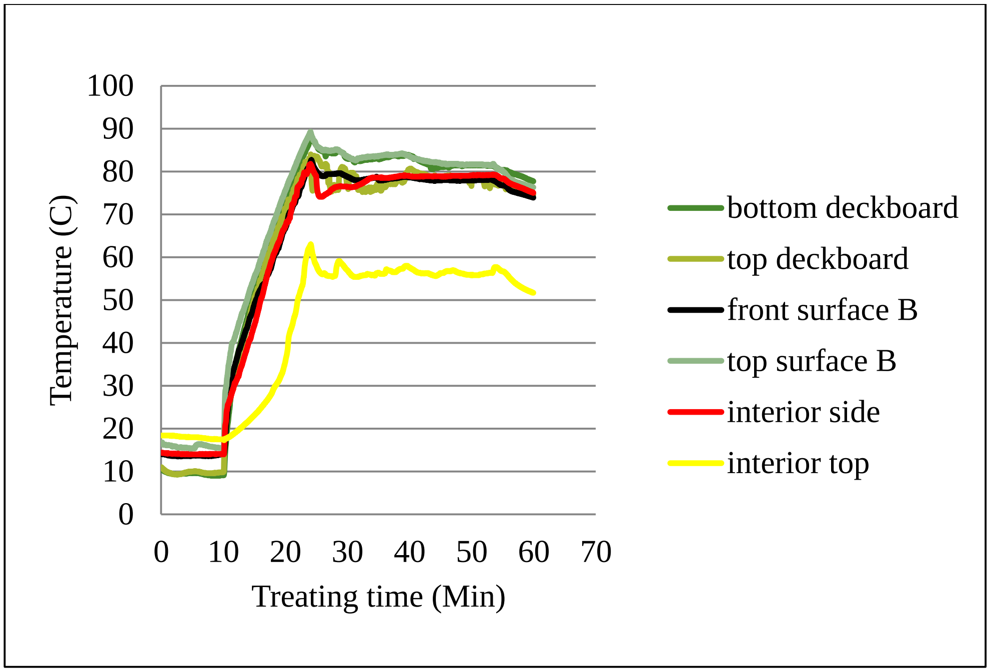


Figure A17. Temperature profile during treatment at the initial vacuum pressure of 100 mmHg at test 17.

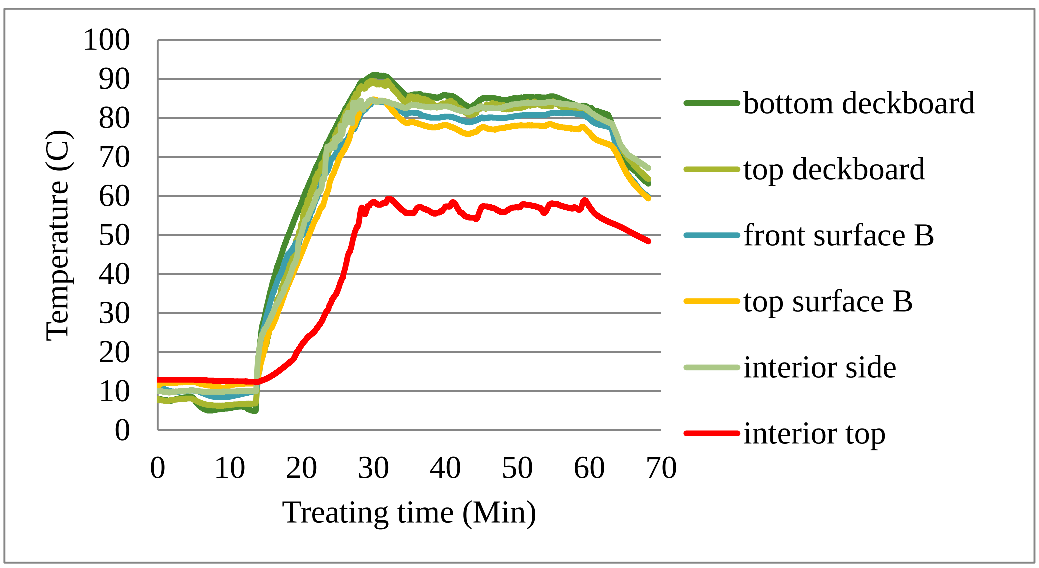


Figure A18. Temperature profile during treatment at the initial vacuum pressure of 100 mmHg at test 18.

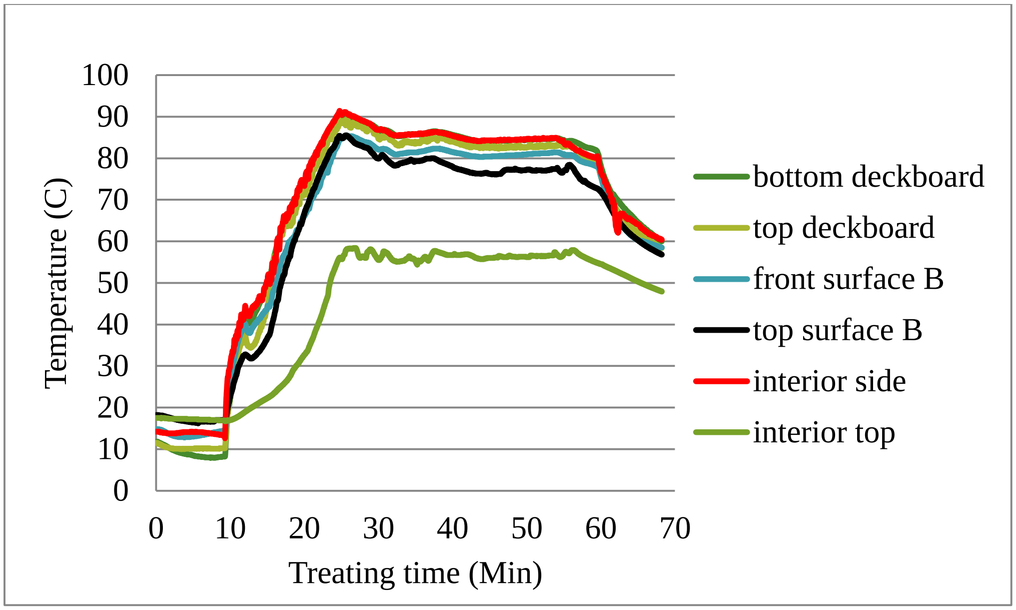


Figure A19. Temperature profile during treatment at the initial vacuum pressure of 100 mmHg at test 19.

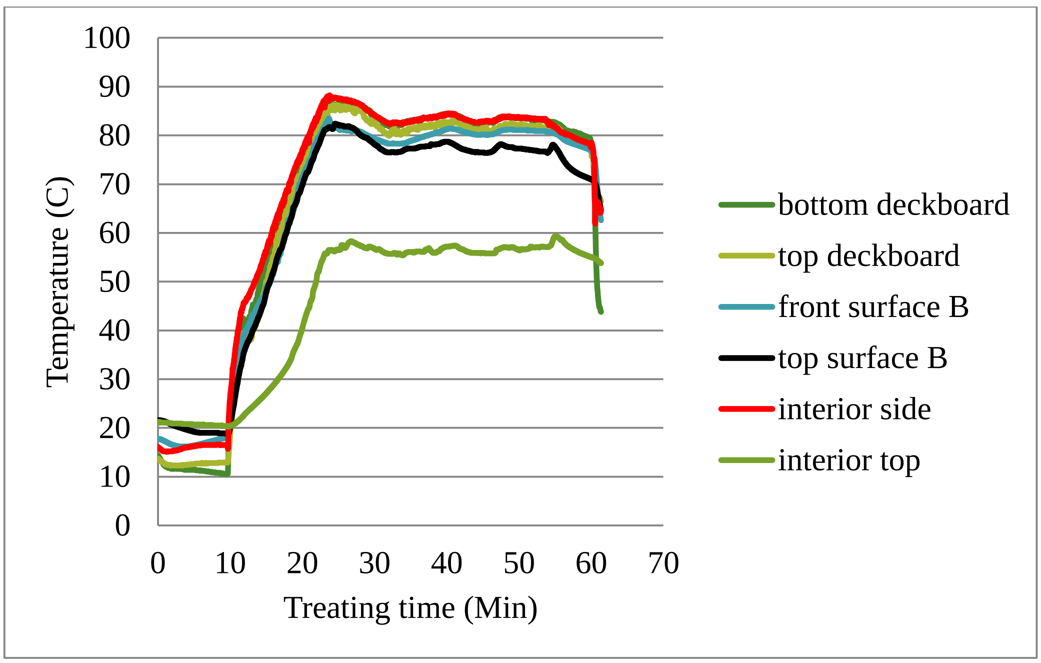


Figure A20. Temperature profile during treatment at the initial vacuum pressure of 100 mmHg at test 20.

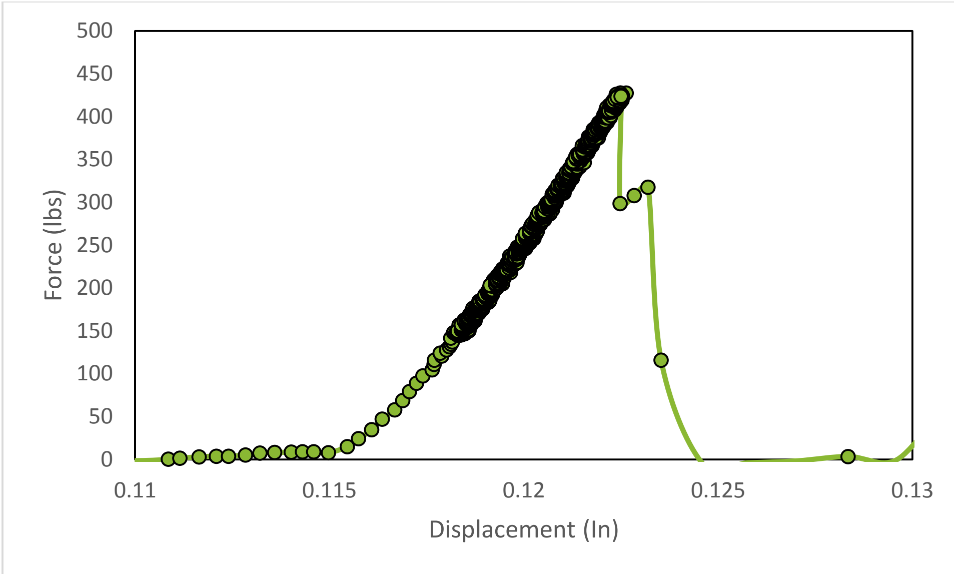


Figure A21. Breaking strength test from the control ceramic tile.

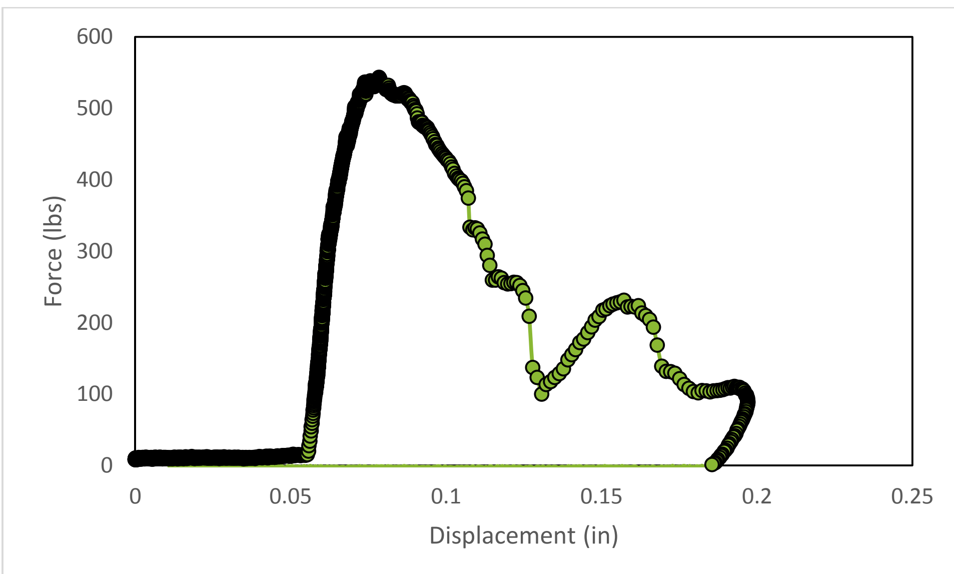


Figure A22. Breaking strength test from the treated marble tile # 101

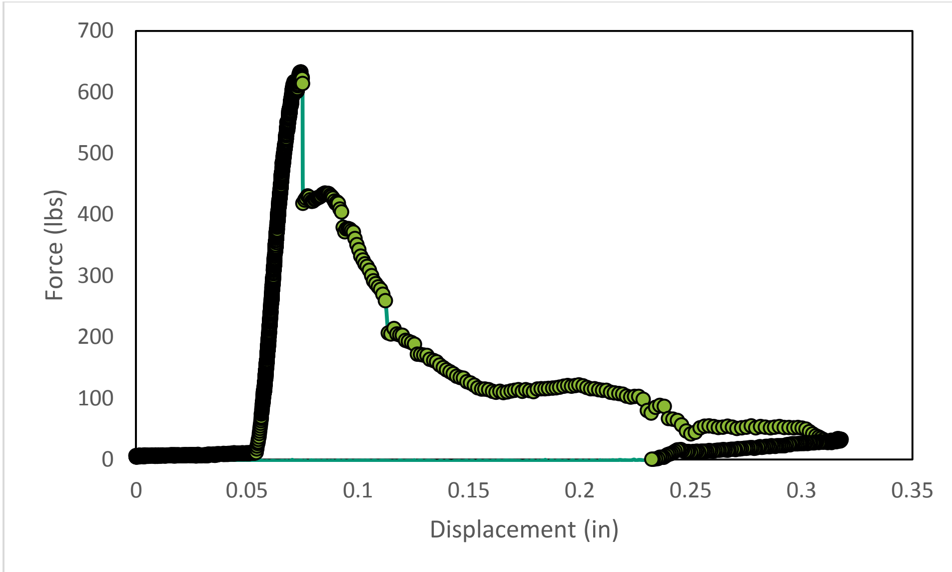


Figure A23. Breaking strength test for the control marble tile.