Research Article

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The effect of transportation vibration on the microbiological status of bottled mineral water

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Abstract

BACKGROUND: Microbiological status and stability are important in mineral waters because of increased global demand. An increase in distribution and supply chains has led to prolonged periods of transportation, causing microbiological changes. Therefore, this study examines the effect of vibration on mineral water quality. Freshly bottled and previously sterilized mineral waters inoculated with microbes isolated from freshly bottled water were tested. The water samples were exposed to random vibration using ASTM (D4169) truck level I, II and III standard vibration protocol for truck transportation at 4 × 1 h at 22 ± 1 °C. After agitation their microbiological status was determined.

RESULTS: Under the influence of low-intensity mechanical impact, the growth rate of autochthonous species in the freshly bottled natural mineral water tripled ($\mu_{control}$ = 0.036 h^{-1}, $\mu_{vibrated}$ = 0.093 h^{-1}) and that of allochthonous species doubled ($\mu_{control}$ = 0.035 h^{-1}, $\mu_{vibrated}$ = 0.069 h^{-1}). The latter was also observed in the case of high-intensity vibration ($\mu_{control}$ = 0.102 h^{-1}, $\mu_{vibrated}$ = 0.200 h^{-1}). The effect of the medium intensity of the standard was manifested in the delay in microbial growth.

CONCLUSION: The impact of transportation vibrations on microbiological status changes in mineral water could be observed when subjected to vibration. The native and allochthonous species of mineral water respond differently to changes in intensity.

Keywords: mineral water; mechanical vibration; microbial status; change in microbe count

INTRODUCTION

There is no life without water and only fresh water is suitable for human consumption. Without food, a person can survive for weeks; however, a 15% loss of water in the body will cause death within a few days. In recent decades, significant growth has been observed in the field of mineral water consumption, not only in Hungary[1,2] but all over the world.[3,4] Global supply chains nowadays deliver bottled mineral water to all parts of the world, so they are no longer just sold in the immediate vicinity of bottling. This growing market is likely to reflect public skepticism about tap water quality,[5] which can be traced back to frequent pollution of urban water supplies, unpleasant taste and odor of tap water, and fluoride and chlorine content of water.[6]

Consumers believe that bottled mineral water does not contain microorganisms and is therefore safer than tap water.[6–8] However, natural mineral waters are not sterile, they have complex ecosystems, and phenotypic and genotypic diversity,[8–10] which are characteristic of a given water extraction site. The physiological and morphological characteristics of aerobic heterotrophic bacteria from different depths of geological strata and sites vary greatly as a function of depth and are markedly different from microbial populations at other depths.[11] In 1974 in Portugal, there was an outbreak of cholera associated with bottled mineral water. *Vibrio cholera* was found in the water source.[12] In 1997 *Campylobacter jejuni* in bottled water was responsible for diseases in Greece.[13] In 2006 in Gran Canaria, *Salmonella enteritica* caused illness in infants, the source of which was bottled water; additionally, microbes were also detected at the bottling plant.[14]

The microbiota of mineral waters can be divided into two major groups: the allochthonous group and the autochthonous group. The allochthon group includes microorganisms entering the environment, but their survival in bottled water is low due to low nutrient content. However, a few weeks after bottling, several researchers isolated pathogenic bacteria from natural mineral water.[15] All microbes present in the environment can enter

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sources, wells and their pipelines. Depending on anthropogenic effects, infectious microorganisms such as *Campylobacter jejuni*, *Escherichia coli*, *Salmonella*, *Shigella* and *Yersinia* may occur.

Most microorganisms living in groundwater are heterotrophic bacteria. Indigenous (autochthonous) microbes such as *Achromobacter*, *Flavobacterium*, *Alcaligenes*, *Acinetobacter*, *cytophages*, *Moraxella* and *Pseudomonas* are the most well known and widespread bacteria in water. Heterotrophic bacteria include pathogens, primarily coliforms (*Escherichia*, *Enterobacter*, *Citrobacter* and *Klebsiella*) and opportunistic pathogens.[16] High heterotrophic microbial counts can cause disease in infants, the elderly, immunosuppressed individuals and pregnant women.

The effect of the distribution of physical circumstances such as vibrations and shocks on the microbiological status of mineral water has a gap in research that needs to be explored and investigated. Therefore, it is essential to understand the impact of transport vibration and its possible human health effects. There are some former studies that investigate the effect of vibration and shock on one liquid food product. The objective of these studies was to report the evidence of vibrations during truck and train transport,[17] and to help food scientists design a more relevant vibration laboratory experiment.[18–21]

In this paper, the focus is on analyzing the changing microbiological status of bottled mineral water under controlled laboratory vibration and temperature conditions.

MATERIAL AND METHODS

Samples

We used two model matrixes. For the first study, freshly bottled mineral water – from a mineral water bottler in northwestern Hungary – was used. The second matrix used a previously sterilized mineral water inoculated with microbes isolated from the examined natural mineral water. Since we have one shaker, 1–1 weeks elapsed between bottling. Manual bottling was done through the sampling tap of the bottling machine. Prior to sampling, the tap was ignited for 10–15 s and then cooled and washed by releasing approx. 2–3 L of mineral water. The bottles, freshly blown from PET preform, were then filled with water from a central jet and sealed with a sterile cap. The water samples were prepared at the plant by the workers.

Vibration experiment setup

For the vibration experiments of this study a well-known and widely used standard vibration standard was employed. This standard, ASTM D4169-16,[22] simulates vibration spectra for road truck transportation. For the vibration simulation, Schedule 'E' PSD profiles of the ASTM D4169-16 standard were used to simulate random vibrations through an accelerated testing procedure with a broadband frequency range of 1–200 Hz. During real transportation this is the frequency band where the vibration has a relative significant intensity. The most intensive vibration response of the cited vehicles parts (generated by road roughness), such as suspension, tires and body structure, can be found in this frequency range.

The vibration test spectra are based on a power spectral density (PSD) plot, which is the fast Fourier transform (FFT) of the random acceleration signal (g). Thus, the feature of movement is expressed by the PSD unit of g^2 Hz^{-1} in that it can be perceived as the power of motion in the function of frequency (Hz). The standard mentioned above recommends three different intensities of PSD plots (low, medium and high level) that simulate vibration circumstances from which all levels were separately applied to this study, in order to observe the effect of various vibration intensities. Although the ASTM vibration standard suggests test times for packaging testing purposes at each intensity level, only a given intensity was used per experiment to simulate the vibration environment. The reason for this is that this study is focusing on the effect of varied vibration intensity and duration on the microbiological status of samples, so the primary aim of the study is not a traditional vibration test of transportation packaging to check packaging integrity. Thus, the PSD plots of the standard were the important and not the recommended test times. Thereby the vibration test times were adjusted to the microbiological experiment and did not perfectly follow the standard test times. Figure 1 shows these PSD plots of the ASTM standard.

The equipment used in this vibration test was an electrodynamic vibration system (TIRA TV59355), which can be seen in Fig. 2, with a climate chamber (Angelantoni AV600C with built-in PT 100 temperature sensor) that was connected to the vibration armature. The latter ensured the temperature remained constant at 22 ± 1 °C during the tests. As preconditioning for test items, another climate chamber (ESPEC PR-3ST with built-in PT 100 temperature sensor) was used for 12 h to ensure that 4 ± 1 °C of samples was achieved prior to the test. 22 °C is optimal for the growth of heterotrophic microbes. Storage at 4 °C was required to slow microbial growth in the samples taken after shaking until the microbiological examination was performed.

A total of 24 master packed units were used in this study. The bottled water samples were tested as follows: for each vibration intensity level (low, medium and high) 6–6 master packed unit (4 × 6 × 0.5 L) bottles were shaken for 4 × 60 min in such way that every hour a master packed unit was removed from the shaker and placed in a climatic cabinet adjusted to +4 ± 1 °C, thus eliminating the temperature exerting an influence on the increase in cell count. The remaining units were kept at 22 ± 1 °C for 12 h, and shaking was repeated. Samples taken from the shaker table were placed in a 4 ± 1 °C climate chamber until transported for microbiological examination. Since the mechanical action was repeated every 13 h (1 h shaking, 12 h rest), the samples were transferred to the microbiology laboratory after the second and fourth shakes.

In each case, the control was natural mineral water (2–2 master packed units) stored at 22 ± 1 °C without shaking and with the same bottling time as the shaken samples. It should be noted that during vibration the samples were not stacked in the climate chamber, in order to avoid the amplification of vibration through stacks.

Microbial counts were performed after bottling, after every 1 h of shaking, at 12 and 24 h after the last shaking, and after an additional 1 and 2 weeks in the inoculated samples.

Microbiological tests

Under the current legislation in force (Min. Decr. 201/2001. (X. 25.)), coliform bacteria and *Pseudomonas aeruginosa* counts, as well as the number of culturable microorganisms at 22 and 37 °C, should be determined in the microbiological diagnostic performed on drinking waters and natural mineral waters. Besides determining the total germ count, in the presence of coliforms the number of *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterococcus* (fecal *Streptococcus*) and sulfite-reducing clostridia should also be determined in a detailed bacteriological examination. Microbiological tests were performed in accordance with the valid legal provision (65/2004. (IV. 27.) FVM-ESzCsM-GKM joint decree). These

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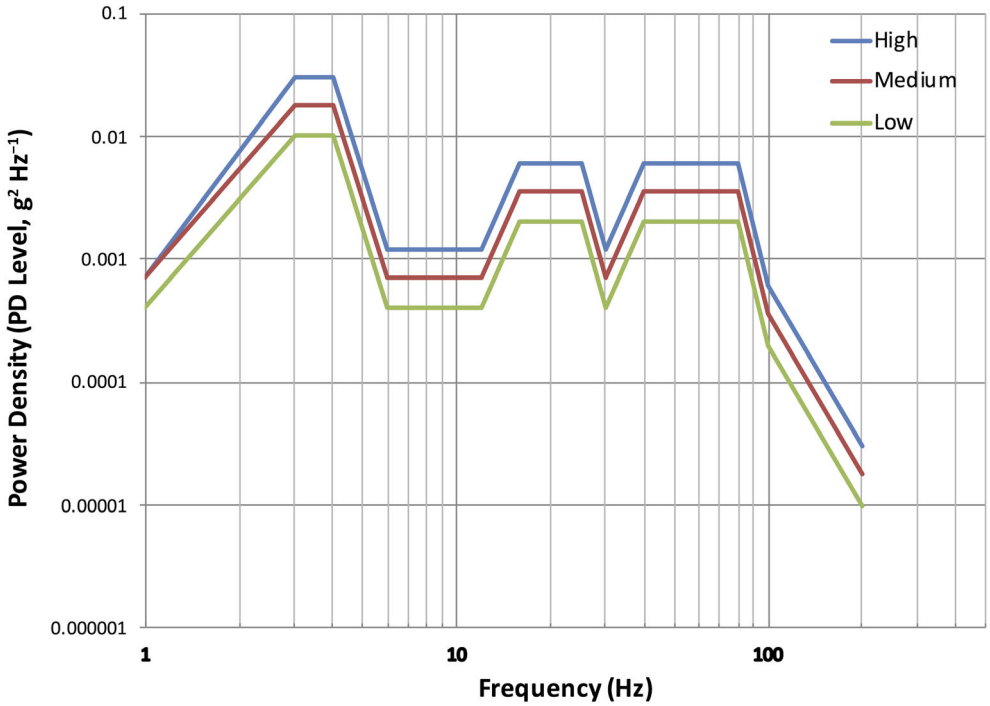

Figure 1. PSD plots of ASTM D4169 standard for random vibration simulations.

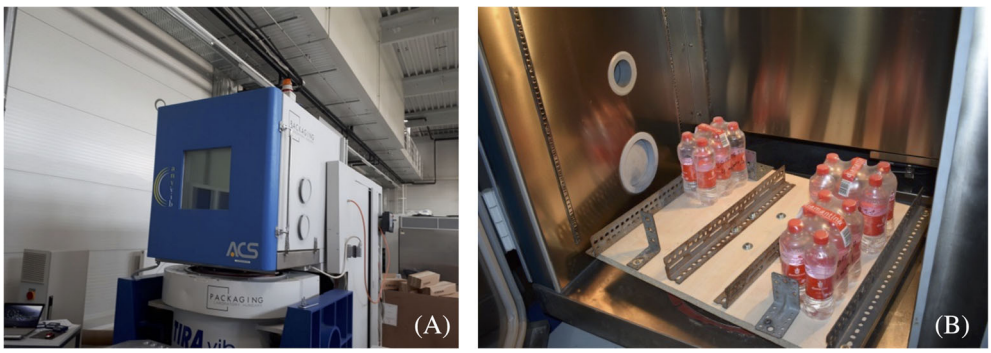

Figure 2. (A) Vibration table and connecting climate chamber. (B) Samples inside the chamber.

counts were performed using classic culturing methods in compliance with relevant standards, as shown in the Table 1.

The pour plate method was used to determine total germ count (Fig. 3(A)), while membrane filtration (Fig. 3(B)) was used to detect the other groups and microbes. The media and the methods, as well the incubation conditions used to detect these microbes, are summarized in Table 2. For membrane filtration tests a Millipore filter stand, vacuum and pressure pump (No. WP6122050), and FilterBio Sterile MCE Gridded Membrane (Labex Ltd, Budapest, Hungary) of 0.22 and 0.45 µm were used.

Table 1. Microbes and standards for their isolation

Microbial group	Standard
Total colony-forming units at 22 and 37 °C	MSZ EN ISO 6222:2000, ISO 6222:1999
Escherichia coli and coliforms	MSZ EN ISO 9308-1:2001
Enterococcus	MSZ EN ISO 7899-2:2000
Number of sulfite-reducing anaerobic (*Clostridium*) spores	MSZ EN 26461-2:1994
Pseudomonas aeruginosa	MSZ EN ISO 16266:2008

Molecular biological testing

To determine the taxonomic classification of the microbes used for inoculation in the second experiment, DNA was purified from freshly bottled water samples from colonies grown on a yeast extract agar plate using Chelex-100 (Bio-Rad, Budapest, Hungary) microbeads, based on the method of Walsh *et al.*[23] During isolation colonies were transferred to a physiological saline solution, and after centrifugation (BioSan Microspin 12; Biocenter Ltd. Szeged, Hungary) (6 min, 11.700 rpm), 200 µL of 10% Chelex-100 solution was added to the pellets and incubated at 100 °C for 10 min with continuous shaking (GrantBio Thermo-Shaker Type: PHMT, Grant Instruments (Cambridge) Ltd, Shepreth, United Kingdom). After incubation, it was centrifuged at 13 000 rpm for 1 min and the supernatant was used for subsequent quantitative

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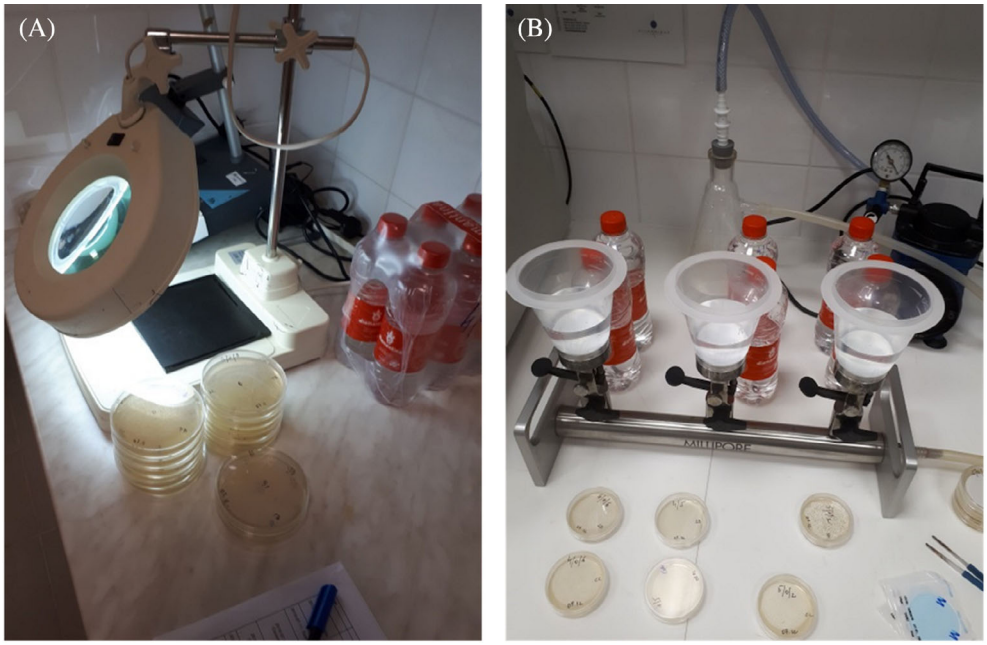

Figure 3. Determination of microbial count by (A) conventional pour plate method and (B) membrane filtration.

Table 2. Media and methods used in the microbiological test

Microbe	Medium (manufacturer)	Method	Incubation temperature (°C)	Incubation time (h)
Total colony-forming units	Yeast Extract Agar (Biolab)	Pour plate	20–22	68 ± 4
Total colony-forming units	Yeast Extract Agar (Biolab)	Pour plate	36	44 ± 4
Escherichia coli and other coliforms	ChromoBio Coliform Agar (Biolab)	Membrane filtration	36	21 ± 3
Enterococcus	Slanezt-Bartley Agar (Biolab)	Membrane filtration	36	44
Enterococcus	Bile Aesculin Azide Agar (Biolab)	Confirmatory test	44 ± 0.5	2
Spore sulfite-reducing anaerobes	Tryptose–Sulfite–Cycloserine–Agar (Merck)	Membrane filtration	36	75 ± 5
Pseudomonas aeruginosa	Cetrimid Agar (Biolab)	Membrane filtration	36	44 ± 4

polymerase chain reaction (qPCR). Purified DNA was amplified with primers commonly used to amplify 16S RNA, yielding a product of almost 1.5 kilobases in length and covering variable regions important for taxonomic classification. The base sequence of the DNA molecule was determined using a service provider with capillary-based Sanger-type sequencing.[24]

Data analysis

For easier illustration of orders of magnitudes, the results of colony counting were subjected to logarithmic transformation. During the evaluation of the results, the obtained data were compared with *F*- and Student's *t*-test using Microsoft Excel 2019. Microbial growth can be divided into four phases: latency (lag), logarithmic (log), stationary and death. In the logarithmic phase, the cell number increases exponentially. From data of the exponential phase, the specific growth rate of the microbes can be determined by the quality of the microbes and the environment. When processing the data, we focused on the exponential phase of microbial growth.

RESULTS AND DISCUSSION

Molecular biological test

The results of the electropherograms were 'blasted' into the sequences of the NCBI database, and clearly a 99–100% agreement with the sequence of *Acidovorax temperans* was obtained.

Microbiological tests

In our studies, neither fecal indicator species (*Pseudomonas aeruginosa*, mesophilic sulfite-reducing spores) was detected in any of the samples.

In the control samples, microbial growth after bottling can be observed in all cases, which has already been reported in other studies.[1,25–27,32]

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Changes in the number of microbes in freshly bottled samples as a function of shaking intensity

Under daily production conditions, the bottling plant fills 1.5 L PET bottles and 19 L plastic containers. The 0.5 L PET bottle formulation was bottled exclusively for the experiment. The different initial germ counts per intensity are due to different bottling times and manual bottling.

In the case of the sample shaken at low intensity, the specific growth rate (μ) increased almost threefold compared to the control when cultured at 22 °C ($\mu_{control,22\ °C}$ = 0.0363 h^{-1}; $\mu_{vibrated,22\ °C}$ = 0.0932 h^{-1}). This stimulating effect also prevailed in the case of saprophytic microbes; the specific growth rate almost doubled due to shaking ($\mu_{control,37\ °C}$ = 0.0347 h^{-1}; $\mu_{vibrated,37\ °C}$ = 0.0692 h^{-1}) (Fig. 4(A)). In the samples shaken at low intensity, microbial growth was already detected in the case of both autochthonous and allochthonous microbes after the first shaking. In the case of the control samples this growth was observed only in the case of autochthonous microbes. In the sample exposed to mechanical action, the autochthonous microbes reached the maximum cell number 12 h earlier and one order of magnitude lower than the control without shaking (max. $\log_{10}$ $cfu_{control}$ cm^{-3} = 5.65, max. $\log_{10}$ $cfu_{vibrated}$ cm^{-3} = 4.63). There is no significant difference in maximal cell numbers.

Compared to the control samples, the increase in the specific growth rate observed in the shaken samples also showed a significant difference at 22 and 37 °C (Table 3). In the control sample microbial growth began after a 12 h latency phase. Rapid proliferation of shaken samples was followed by a latency period after the first shaking and then continued at the rate observed at the start of the study after cessation of agitation. No difference was found in the number of maximum colony-forming units.

Examining the effect of mechanical agitation and microbial growth at medium intensity according to ASTM D4169-16, microbial growth was observed only after its cessation at both 22 and 37 °C (Fig. 4(B)). In the case of autochthonous microbes, the onset of microbial count growth was observed 12 h after mechanical agitation, and in the case of the contaminating flora 24 h later. There was no significant difference in the specific growth rate compared to the control sample in the shaken samples (Table 3).

Autochthonous microbes reached a maximum germ count of two orders of magnitude lower than in the control sample due to shaking, but there are 10^4 orders of magnitude (max. $\log_{10}$ $cfu_{control}$ cm^{-3} = 6.19, max. $\log_{10}$ $cfu_{vibrated}$ cm^{-3} = 4.17). For allochthonous microbes the difference is only an order of magnitude (max. $\log_{10}$ $cfu_{control}$ cm^{-3} = 0.90, max. $\log_{10}$ $cfu_{vibrated}$ cm^{-3} = 1.32). In addition, a stimulatory effect of shaking

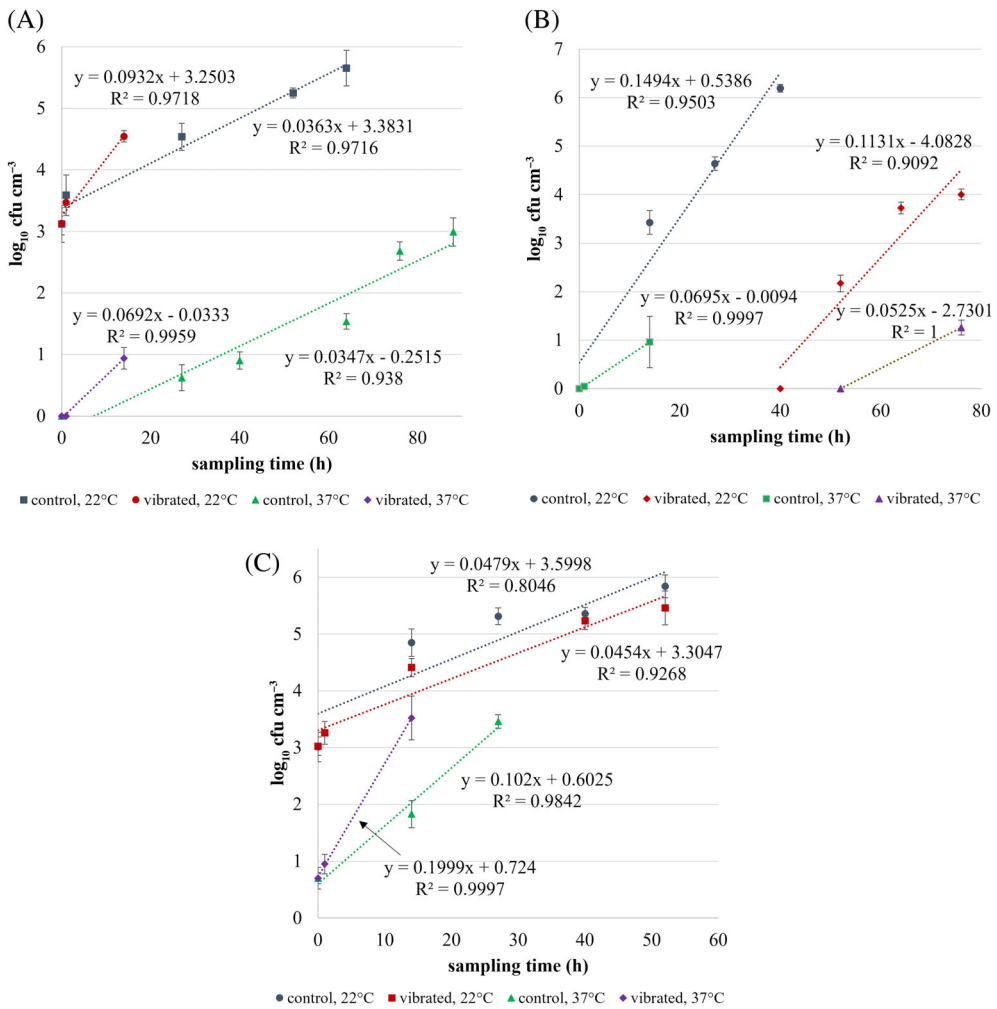

Figure 4. Change in the total germ count of the control and (A) low-intensity, (B) medium-intensity and (C) high-intensity shake of freshly bottled mineral water samples during the exponential phase of growth culturing at 22 and 37 °C.

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Table 3. Differences in specific growth rates (μ) for the exponential phase of microbial growth in freshly bottled mineral water

	Intensity of vibration			
Temperature (°C)	Low	Medium	High	Control
22	0.093 ± 0.002ac			0.036 ± 0.003b
		0.113 ± 0.005ad		0.149 ± 0.001a
			0.045 ± 0.004ac	0.048 ± 0.004a
37	0.069 ± 0.418ae			0.035 ± 0.001b
		0.053 ± 0.008af		0.035 ± 0.019a
			0.020 ± 0.034ag	0.102 ± 0.011b

Values with different exposures (a,b) differ significantly ($P < 0.05$) within rows.
Values with different exposures (c–g) differ significantly ($P < 0.05$) within columns.

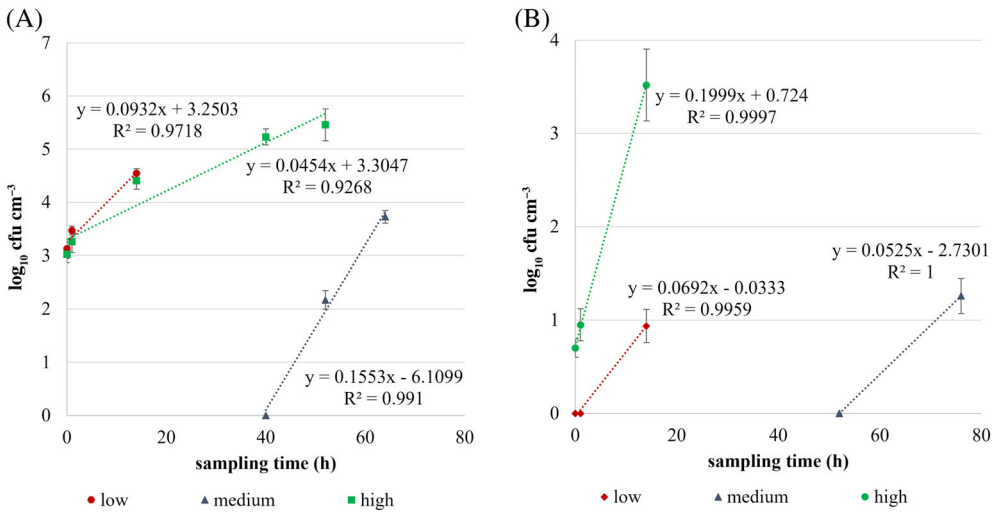

Figure 5. Changes in the microbial count of freshly bottled minerals, shaken at different intensities and culturing at different temperatures – (A) 22 °C; (B) 37 °C) – during the exponential phase of reproduction.

on the specific growth rate was observed ($\mu_{control,37\,°C} = 0.069\ h^{-1}$; $\mu_{vibrated,37\,°C} = 0.052\ h^{-1}$). In the sample shaken at high intensity (Fig. 4(C)), no difference in the growth rate of autochthonous microbes was observed compared to the control ($\mu_{control,22\,°C} = 0.047\ h^{-1}$; $\mu_{vibrated,22\,°C} = 0.045\ h^{-1}$). The growth of the contaminating flora was significantly accelerated by this intensity (Table 3) compared to the control; the specific growth rate doubled ($\mu_{control,22\,°C} = 0.102\ h^{-1}$; $\mu_{vibrated,22\,°C} = 0.199\ h^{-1}$). The allochthonous species reached the maximal cell number 12 h earlier than the control; however, no order of magnitude difference was observed (max. $\log_{10}$ $cfu_{control}$ cm^{-3} = 3.46, max. $\log_{10}$ $cfu_{vibrated}$ cm^{-3} = 3.78).

We compared the specific growth rate observed for samples shaken at different intensities. (Fig. 5(A,B)). In the case of allochthonous microbes, in addition to shaking at low and medium intensities, we found a significant difference in the specific growth rate ($\mu_{low,22\,°C} = 0.093\ h^{-1}$; $\mu_{medium,22\,°C} = 0.155\ h^{-1}$) (Table 3). This difference is not observed when comparing low–high ($\mu_{low,22\,°C} = 0.093\ h^{-1}$; $\mu_{high,22\,°C} = 0.045\ h^{-1}$) and medium–high ($\mu_{medium,22\,°C} = 0.155\ h^{-1}$; $\mu_{high,22\,°C} = 0.045\ h^{-1}$) intensities. A significant difference in the growth of autochthonous microbes was observed at all three intensities (Table 3). The highest specific growth rate was observed at high intensity ($\mu_{high,37\,°C} = 0.199\ h^{-1}$), while the lowest was observed at medium intensity ($\mu_{medium,37\,°C} = 0.052\ h^{-1}$).

However, it should also be taken into account that in the case of the sample shaken at medium intensity, due to the initial low germ count, microbial growth started only after the mechanical effect ceased (Fig. 5(A,B)). Rapid microbial growth after bottling was delayed due to vibration. The initial higher germ count (at low and high intensity) can be traced back to the conditions

Table 4. Comparison of the specific growth rate of autochthonous and allochthonous microbes in the case of freshly bottled natural mineral water *versus* shaking intensity

	μ ± SD	
	Temperature of culturing	
Intensity of vibration	22 °C autochthonous microbes	37 °C allochthonous microbes
Low	0.093 ± 0.002a	0.069 ± 0.418b
Medium	0.113 ± 0.002a	0.053 ± 0.008b
High	0.045 ± 0.004a	0.020 ± 0.034b

μ, specific growth rate.
Values with different exposures (a,b) differ significantly ($P < 0.05$) within rows.

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Table 5. Differences in specific growth rates (μ) for the exponential phase of microbial growth in inoculated mineral water

	Intensity of vibration			
Culturing temperature (°C)	Low	Medium	High	Control
37	0.130 ± 0.003ac			0.186 ± 0.008b
		0.081 ± 0.003ad		0.166 ± 0.003b
			0.062 ± 0.006ae	0.186 ± 0.008b

Values with different exposures (ab) differ significantly ($P < 0.05$) within rows.
Values with different exposures (c–e) differ significantly ($P < 0.05$) within columns.

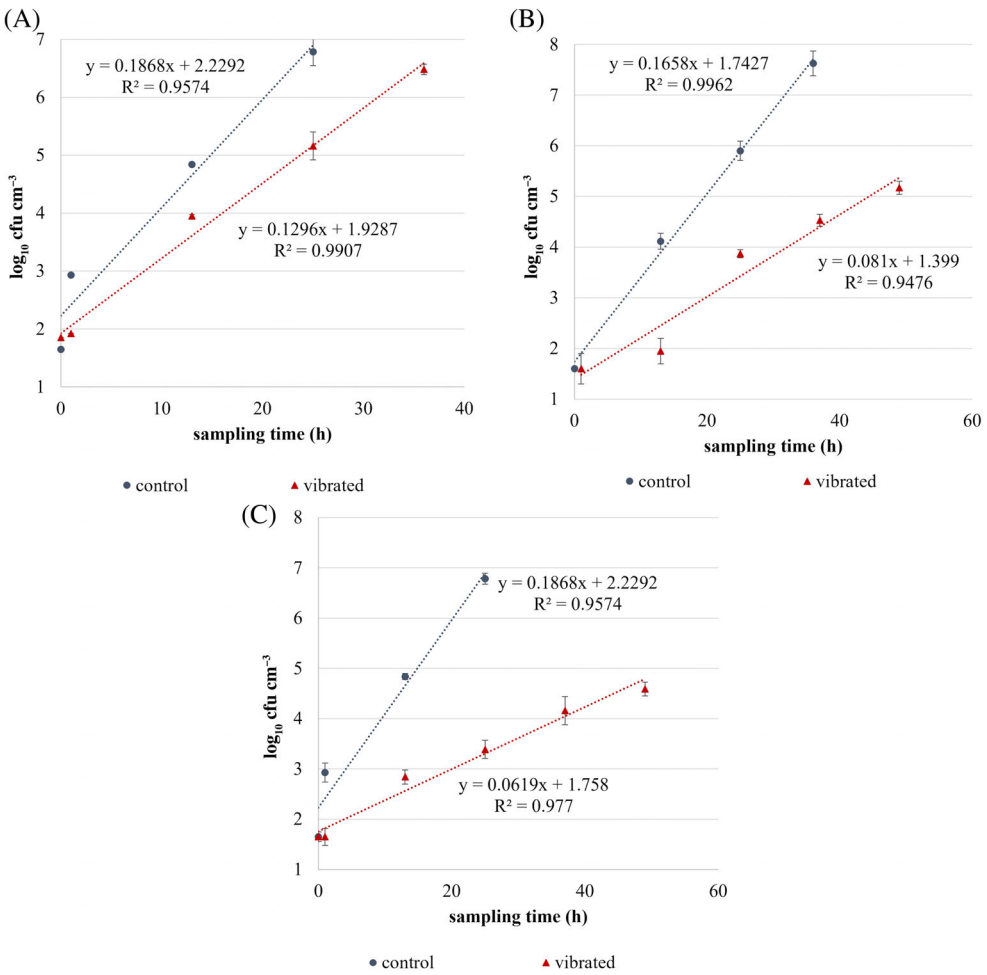

Figure 6. Change in the total germ count of the control and (A) low-intensity, (B) medium-intensity and (C) high-intensity shake of inoculated mineral water samples during the exponential phase of growth culturing at 37 °C.

of manual bottling. The initial low germ count does not mean that there are no microbes in the water at all, but rather they are below the limit of detection and/or present in VBNC (viable but not culturable) form.

The maximum germ counts cannot be compared due to the different values of the initial sample, but it is striking that in the sample shaken at medium intensity the autochthonous microbes reached the order of 10^4 within 24 h after the cessation of the mechanical action. One order of magnitude difference in the increase in cell number was detected at low intensity and 2 orders of magnitude at high intensity.

At low and medium intensity, the autochthonous microbes multiplied faster, while at high intensity shaking the allochthonous microbes multiplied faster, and the difference in the specific growth rate of autochthonous and allochthonous microbes was significant at all three intensities of ASTM D4169-16. (Table 4).

Changes in the number of microbes in inoculated samples as a function of the intensity of mechanical agitation

Acidovorax temperans belongs to the genus *Acidovorax*, which has recently been identified or reclassified from the genus

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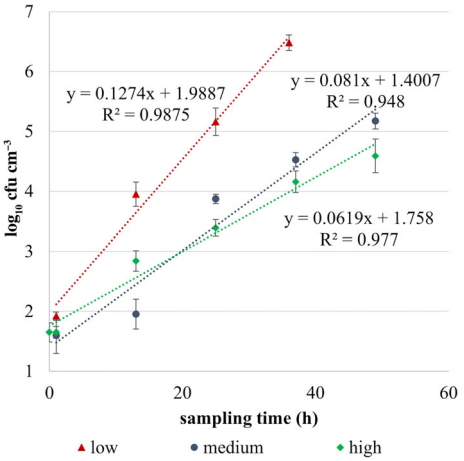

Figure 7. Changes in microbial count of inoculated waters, shaken at different intensities during the exponential phase of reproduction.

Pseudomonas.[28] It grows at 37–42 °C, as Willems *et al.*[29] found; therefore, the culture after shaking was analyzed at this temperature.

Compared to the control, the specific growth rate for the shaken samples showed significant differences (Table 5) at all three intensities. As the intensity of mechanical agitation increased, the specific growth rate decreased (μ_{low} = 0.129 h^{-1}, μ_{medium} = 0.081 h^{-1} and μ_{high} = 0.062 h^{-1}) (Fig. 6(A–C)), and this decrease was also significant between the individual intensities (Table 5). The same decrease was observed in the studies at different intensities in terms of the maximum number of cells achieved (max. $\log_{10}$ cfu $cm^{-3}{}_{low}$ = 6.48, max. $\log_{10}$ cfu $cm^{-3}{}_{medium}$ = 5.4 and max. $\log_{10}$ cfu $cm^{-3}{}_{high}$ = 4.83).

The samples reached the maximum cell number at all intensities during the exposure (0–50 h); the rates of growth were 3–4 orders of magnitude. *Acidovorax temperans* multiplied rapidly with low-intensity shaking (Fig. 7).

Due to there being a mixed microbial population in natural mineral waters and only one bacterial species was used for inoculation in our studies, we examined whether there was a difference in the shake-induced growth rate change between the two matrices. Since the growth of *Acidovorax temperans* was studied at 37 °C, the comparison was also performed with the same data of freshly bottled mineral water.

A significant difference in specific growth rate was observed at all three intensities between the mixed culture and the monoculture (Table 5). In the case of monoculture, microbial growth in the inoculated sample had already started at the beginning of the study (Fig. 8), while in the case of freshly bottled natural mineral water, as a consequence of the mechanical effect performed at

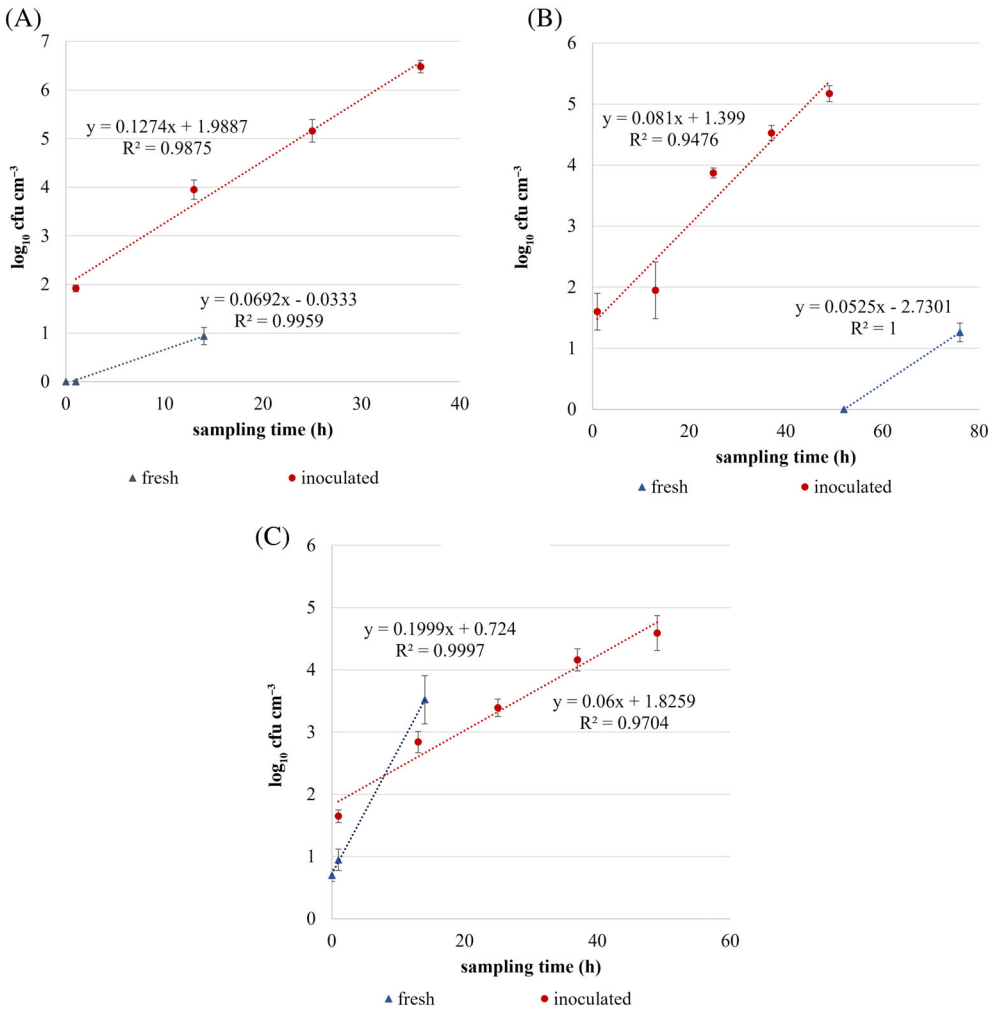

Figure 8. Comparison of the exponential stage of reproduction at different intensities – (A) low; (B) medium; (C) high) – of vibration based on the results of microbial cultivation of freshly bottled natural mineral water and previously sterilized natural mineral water inoculated with *Acidovorax temperans*.

Table 6. Differences in specific growth rates (μ) for the exponential phase of microbial growth in freshly bottled and inoculated mineral water

	Intensity of vibration					
	Fresh			Inoculated		
Temperature of culturing (°C)	Low	Medium	High	Low	Medium	High
37	0.069 ± 0.013a			0.127 ± 0.005b		
		0.053 ± 0.008a			0.81 ± 0.003b	
			0.200 ± 0.034a			0.062 ± 0.006b

Values with different exposures (a,b) differ significantly ($P < 0.05$) within rows.

medium intensity, it could be observed only after the cessation of agitation (Fig. 8(B)).

While performing vibrations at the high intensity of ASTM-D4169, we observed that the specific growth rate of the allochthonous species was higher in the freshly bottled natural mineral water, which contains different species (μ_{fresh} = 0.199 h^{-1}, $\mu_{inoculated}$ = 0.052 h^{-1}) compared to the inoculated sample. (Table 6; Fig. 8(C)).

DISCUSSION

In our experiment, the decrease in specific growth rate and maximum cell number observed in the inoculated samples can probably be traced not only to the intensity of the mechanical effect, but also to the time-accelerated nature of the applied simulation. Duration of transport may in fact be longer than in an applied simulation; this is due to the characteristics of PSD curves in vibration tests using artificial power, as this amplification function creates so-called time acceleration.[30] Furthermore, the load configuration and packaging system can also have an effect on the gain with their different vibration response intensity, or a real road test (without artificial amplified vibration intensity or at lower intensity) may have a milder effect.

The initial low germ count of autochthonous microbes a few days after bottling,[11,31,32] 1–3 weeks based on studies by other authors,[9,10,30] can reach 10^4–10^6 cfu cm^{-3}. Our samples reached a maximum germ count of 10^4–10^6 cfu cm^{-3} within 24–48 h.

The reason for the change in germ count after bottling is not yet clear. Non-carbonated mineral water generally contains more microbes than carbonated waters.[11,33] The rapid increase in bacterial count after bottling is due to the oxygen supply to the water, the increase in temperature and the amount of nutrients present in the bottle.[34,35] Due to the limited availability of nutrients in the water base, the bacteria that make up the natural microbiota of mineral waters are often starved. As a result, their morphology may change, so that the shape of the cells may deform by taking on an egg shape, and their diameter may decrease to such an extent that they pass through the standard 0.45 µm or, according to some literature, even membrane filters with a pore diameter of 0.2 µm.[11] Previous publications draw attention to the fact that 0.1–1.0% of microbes occurring in the natural environment can be detected by conventional methods. This is due to the low nutrient content. Most microbes are in a living but not culturable (VBNC) state. These microorganisms cannot be detected by conventional culturing methods (pour plate method, membrane filtration).

The evaluation should take into account that increases in cell numbers after zero do not mean that the sample did not initially contain live cells, but that they were present in numbers below the detection limit of the standard assay.

CONCLUSIONS

As a result of our studies, we can conclude that the low-intensity mechanical agitation accelerates the specific growth rate of both autochthonous and allochthonous microbes in freshly bottled mineral water.

Although mechanical action at medium and high intensities did not cause a significant change in the maximum germ count of freshly bottled mineral water, the specific growth rate of allochthonous species was doubled at high intensity. The increase in the number of allochthonous species in bottled mineral water is clearly detrimental to the health of consumers, also taking into account that the number of some species in the water may stabilize for up to half a year or more before it begins to decline.

The interaction between microorganisms in natural mineral water may also explain the differences in proliferation and cell counts between freshly bottled and contaminated mineral water due to shocks of different intensities.[32]

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