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Is the sound an ally against microplastics?

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ABSTRACT

The presence of small plastic particles, also known as microplastics, in freshwater is a current issue that poses a significant environmental problem and detecting them effectively would be an important step towards finding a solution. Various methods have been used so far to detect them, such as spectrophotometry, chelating agents, etc., but no one has detected them through sound. Using river water collected from different points in Pamplona: in Olloki (before entering the city), in Club Natación Pamplona (through the city centre) and in Ororbia (after the city); with a speaker, a tuner and some laboratory instruments, a simple and useful procedure has been created to find microplastics in the water, not without conducting several controls to ensure that plastic is truly being measured and that the research is not influenced by other agents such as sediments, stones, biodiversity, salt or bottle sampling materials.

KEYWORDS

Microplastics, freshwater, sound, detection, musical notes.

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BIOGRAPHY

Lucía Royo Asa was born in Pamplona, Navarra. She is 16 years old and has completed the fifth year of Secondary School at Colegio Luis Amigó. She enjoys watching series and hanging out, and her favourite hobbies are cooking, reading and writing. Thanks to her passion for writing, she has participated in contests such as *Inspiraciencia*, where she was selected as a finalist with her story "Star War" in 2019.

Ainara San Miguel Rodríguez is also 16 years old. She is from Spain and currently lives in Pamplona, Navarra. She has been studying at Colegio Luis Amigó for 14 years and is currently in her fifth year of Secondary School in the Science option. In her free time, she enjoys playing basketball and listening to music.

Lucía and Ainara are two students who love conducting research. They both joined the Robotic and Scientific Investigations Club, called Curiosity, at their school in the 2021-2022 academic year. There, they started developing this project along with their classmate Jorge Bosque. As part of their work, they created a video that was selected as a finalist in the *Ciencia Clip* contest. As a result, the team had the opportunity to visit Bilbao and attend the Awards Ceremony organized by *Naukas*.

Initially, this investigation was carried out as a final project for the Biology subject in the fourth year of Secondary School. However, Ainara and Lucía continued to develop the project beyond that and are still working on it today.

INTRODUCTION

Since the term "microplastic" was introduced in 2004 by marine biologist Richard Thompson, Director of the Marine Institute at the School of Biological and Marine Sciences, Faculty of Science and Engineering, University of Plymouth (University of Plymouth, n.d.), they have been found in countless places, from water in seas and poles to everyday food. Between 60 and 80% of marine debris is plastic, especially microplastics, which are small plastic fragments with a diameter smaller than 5 mm. Like regular plastic, they take over 500 years to decompose (Greenpeace, n.d.).

Although most studies have been conducted on saltwater, there is also contamination of microplastics in freshwater. It is important to remember that both freshwater and saltwater environments have ecosystems with thousands of species that predominantly communicate through sound waves. Sound travels faster in water than in air, and different animals, such as dolphins, emit whistles and chirps that travel at a frequency of 300 KHz (Bazúa-Durán, 2010).

The water analyzed in this experiment comes from the Arga River, the main river of the city of Pamplona in Navarra, Spain. It originates in the Quinto Real mountains, where it is regulated by the Eugui reservoir (with a capacity of 21 Hm^3 of water), the main reservoir of the city. Upon entering Pamplona, the river takes a meandering course with a floodplain ranging from 500 to 1500 meters and its hydrological regime exhibits spectacular floods. The Arga is a dynamic river, with erosion occurring on the outer side of the meanders and sedimentation on the inner side. However, this dynamism is heavily altered and almost eliminated by the presence of infrastructure, buildings, and urban uses in the city (orchards, sports clubs, etc.), so its course can be considered fixed (Ayuntamiento de Pamplona, n.d.).

Thanks to the data provided by Ismael Pérez Mata, from the Department of Rural Development and Environment of the Government of Navarra, including the 8 water samples taken from the Arga River during 2022 and the historical data from the sampling point as it passes through Pamplona, the water can be classified as moderately hard, with light mineralization and calcium bicarbonate content (Gobierno de Navarra, 2023). Furthermore, between the two flow measurement stations in the city (located 8.2 km apart) with a flow rate of 14.38 m^3/s, the travel time of the same peak between them was 130 minutes between April 22 and April 24, 2023, as shown in Figure 1 in the appendix (SAIH Navarra, 2023). These data will be used to calculate the river velocity on the sampling day and the waiting time between the selected sampling points for this work.

On the other hand, it is also important to note that for the experiments in this research, a digital keyboard will be used to play four different notes (C, D, E and G) belonging to the pentatonic scale of C major. Generally, musical scales tend to vary depending on the instrument that plays them. That is why different musical scores are created for the same song based on the instrument being used. However, the pentatonic scale is shared between the guitar and the piano. Given the collaboration of Jorge Bosque in this project, who also plays the guitar, and the knowledge of Lucía Royo, who studied piano for 4 years, it was decided to use the pentatonic scale of C major to analyze the results perfectly.

This scale is characterized by omitting the semitones of the natural scale (F and B). The pentatonic scale of C major consists only of notes separated by fifths (C, D, E, G and A). Fifths refer to the musical intervals between two notes whose frequencies are separated by a ratio of 9/8 or 32/27, generally. From this information, we can calculate the frequencies and subsequent notes of this scale (ETS. Ing. de Telecomunicaciones, 2006).

For the execution of this experiment, we will start from middle C (C4) and follow a pentatonic order: C4 (261.63 Hz), D4 (293.67 Hz), E4 (329.63 Hz), and G4 (391.99 Hz) (Wikipedia, 2022). Additionally, the same notes will be used in a higher octave (octave 5, natural scale) since higher-pitched sounds are transmitted at higher frequencies than lower-pitched sounds, which will be helpful for obtaining results (Academia Solfeando, 2023). Therefore, the notes C, D, E and G will be used again, but this time with higher frequencies: 523.25 Hz (C5), 587.33 Hz (D5), 659.26 Hz (E5) and 783.99 Hz (G5) (Wikipedia, 2022).

After speaking with Eva Asa Olivares, a music teacher at the Batucklang-Asa academy and a clarinetist in the city of Pamplona's marching band, *La Pamplonesa*, it was confirmed that the use of the Anglo-Saxon musical notation is internationally accepted. This system is based on an alphabetical base equivalent to the Latin nomenclature of notes: C (Do), D (Re), E (Mi), F (Fa), G (Sol), A (La) and B (Si). Depending on the octave in which these notes are, they will be accompanied by the corresponding number, which should be subscripted in the case of the Franco-Belgian naming convention (Do, Re, Mi, Fa, Sol, La and Si) or normal numbers in the case of the Anglo-Saxon notation.

Lastly, it is worth highlighting the Sustainable Development Goals (SDGs) in which this research is framed. Specifically, goal number 6 (Clean Water and Sanitation) with target 6.3: Improve water quality by reducing pollution; goal number 13 (Climate Action) with target 13.2: Integrate climate change measures into national policies, strategies, and planning; and goal number 14 (Life Below Water) with target 14.1: Prevent and significantly reduce marine pollution of all kinds (UN, 2015).

All of this, combined with our concern for the environment and pollution, along with our musical interests and knowledge, have been the key factors that have led us to undertake this project. We believe that combining music and science in a research project can be both fun and useful for testing the presence of microplastics in water.

PURPOSE

After this initial analysis, the following main purpose is proposed: Could the presence of microplastics in freshwater be detected by analyzing sound transmission?

This research aims to achieve the following specific purposes:

1. Perform a negative control using distilled water to verify that the tuner works correctly in the absence of any dissolved components.
2. Test how sound is transmitted in water through the following positive controls:
    - 2.1. Control with macroplastics.
    - 2.2. Control with microplastics.
    - 2.3. Control with the metal bottle used for sampling.
    - 2.4. Control with the salinity concentration of the Arga River based on the data provided by the Government of Navarra.
    - 2.5. Control with organic matter from the river.
    - 2.6. Control with sediments from the river.
    - 2.7. Control with stones from the river.
3. Analyze whether the presence of microplastics in river water can be measured before, during and after its passage through the city of Pamplona:
    - 3.1. Samples from Olloki (before passing through the city).
    - 3.2. Samples from the Club Natación footbridges (at its passage through the city).
    - 3.3. Samples from Ororbia (after passing through the city).

STATE OF THE ART

Nowadays, the number of articles and experiments related to this research is not very abundant. Almost all the articles found indicate the use of spectrophotometry as a technique for measuring microplastics in water. However, this option is not viable in this work due to the high prices of a spectrophotometer, ranging from €3,000 to €9,000 (Labotienda, 2022).

Another option found is the use of a chelating agent to trap microplastics for subsequent quantification (Da Costa *et al.*, 2021), but it is economically inaccessible too (Biovea, 2022).

Using these techniques, Spanish scientists have concluded that the microplastics found in freshwater in the Arctic come from the textile industry, although small particles from household products such as toothpaste and exfoliants are also present (González-Pleiter *et al.*, 2020). However, no reference has been found indicating that any research project has analyzed the presence of microplastics in water through sound.

Nevertheless, ultrasound has been used in quantification and inactivation techniques of microorganisms, such as those involved in the acidogenic fermentation of wastewater (Sarkar *et al.*, 2023), those surviving food processing treatments (Shao *et al.*, 2023) and fungi that contaminate food with their toxins (Hashemi-Moosavi *et al.*, 2021). These uses in the microscopic world may indicate the beginning of the application of sound for the study of microplastics.

MATERIALS AND PROCEDURE

First of all, to carry out the sampling, the locations were selected where water from the Arga River would be collected as it passes through the city of Pamplona at three different points: before reaching the city, in Olloki (42° 50' 00.1" N - 1° 35' 12.0" W), where the river is narrow and the current is strong; passing through the city of Pamplona, at the Club Natación Pamplona footbridges (42° 49' 05.8" N - 1° 38' 10.4" W), where the river is wider and the current is calmer; and finally, at the exit of the city in Arorbia (42° 48' 51.5" N - 1° 44' 55.2" W), where the river is slightly narrower and the current is calm.

Using the data provided by Ismael Pérez Mata, from the Department of Rural Development and Environment of the Government of Navarra, the average velocity of the river was calculated to be 3.8 km/h, without considering factors such as obstacles in the channel, slope or the addition of tributaries, which can alter the velocity. Additionally, using the IDENA web application (Government of Navarra, n.d.), the distance in kilometers between the sampling locations was calculated, as shown in Figures 2 and 3. With all this data, the approximate waiting time to take samples from the same water was calculated. The following were the waiting times:

- Between Olloki and the Club Natación footbridges (7.99 km): 2 hours and 6 minutes.
- Between the Club Natación footbridges and Arorbia (15.29 km): 4 hours and 1 minute.

The total waiting time was 6 hours and 7 minutes, which was accurately measured using a chronometer.

To collect water from the river, it was stored in three 0.5 L metal bottles (up to a total of 1.5 L per sampling location). The water remained in the metal bottles for no more than 30 minutes, after that it was transferred to glass containers upon arrival at the laboratory for immediate analysis. This time was also measured using a chronometer. In addition to the river samples, organic matter, sediments, and stones were collected at the three points for subsequent positive controls. The water temperature was measured using a submersible digital thermometer.

The instruments used in the different analyses were a scale, a graduated cylinder, a crystallizer, a hotplate, a submersible digital thermometer, a beaker, a waterproof speaker (minimum IPX7 or IP67 rating, capable of withstanding water splashes) and a tuner (Guitar chromatic turner Korg CA-30). The notes were played using a mobile phone through the web (Musicca, 2023) and the entire process was recorded using an iPad and a tripod.

1. Negative control with distilled water:

For the negative control, 280 mL of distilled water were measured using a graduated cylinder and poured into the crystallizer, then heated on the hotplate until reaching 25°C. Once the water reached the desired temperature, it was poured into a beaker that previously contained the speaker, completely covering it. Next, with the speaker connected to the mobile device via Bluetooth, the following notes were played from the digital piano website: C4, D4, E4, G4, C5, D5, E5 and G5 (played in the order listed). The frequency oscillations of the emitted notes were measured with the tuner and recorded with the iPad and tripod for subsequent analysis of the results.

2. Positive controls:

Positive controls were carried out with: macroplastics, microplastics, metal from the bottle, salt concentration, organic matter, sediments and stones to verify whether they affected the transmission of the notes or not. The experiment was the same as described above, with the variations described below.

2.1. Macroplastic control:

A plastic bottle was cut into 2 g of visible pieces, 1 cm^2 approximately each one, and they were added to 280 mL of previously heated distilled water in the crystallizer with the submerged speaker. The plastic pieces should surround the speaker on all sides.

2.2. Microplastic control:

To contaminate this microplastic control, 2.5 g of an exfoliating gel (facial exfoliant gel of The Himalaya Drug Company) was used, which was dissolved in 280 mL of distilled water at 25°C before pouring it into the crystallizer with the speaker inside.

2.3. Control of the metal bottle for sample collection:

Water was transported from the sampling locations to the laboratory in metal bottles, and to check if the material of the bottle contaminated the samples, thus affecting the sound transmission, this control was performed. A time of 30 minutes was set between collecting the river water inside the bottle and the start of the experiment in the laboratory. Using a chronometer, the time was verified. To perform this control, 280 mL of distilled water were left in the same metal bottles used for sample collection for 30 minutes. Then, the experiment was realized as previously described with this water.

2.4. Control of river salt concentration:

According to the data provided by Ismael Pérez Mata, from the Department of Rural Development and Environment of the Government of Navarra, the concentration of $Ca(HCO_3)_2$ in the Arga River as it passes through Pamplona is around 225.9 mg/L. With this information, the concentration that would be present in 280 mL of distilled water (the amount of water needed for the control) was calculated: 0.063252 g. This amount of compound was weighed using a precision scale and dissolved in distilled water. Then, the experiment was conducted as in previous controls.

2.5. Control with organic matter:

At each sampling point, a sample of organic matter from the river (leaves, algae, microorganisms, etc.) was collected to carry out this control. 16.7 g of organic matter were weighed and mixed with 280 mL of distilled water that had been previously heated to 25°C and poured into the crystallizer with the speaker inside, surrounding it. One control was performed for each collection point.

2.6. Control with sediments:

Sediments were also collected from the riverbanks or bottom at each sampling point. For the control, 21.1 g of sediments were mixed with 280 mL of previously heated distilled water at 25°C in the crystallizer with the speaker inside, surrounding it. One control was performed for each collection point.

2.7. Control with stones:

This analysis was very similar to the previous two, using 138.5 g of stones of varying sizes. The stones were placed in the crystallizer, once the speaker was placed, surrounding it, and before adding 280 mL of distilled water heated to 25°C. One control was performed for each collection point.

3. River samples:

Five measurements were taken for each of the points where water was collected from the Arga River (Olloki, Club Natación footbridges and Ororbia). For the experiments, 280 mL of river water were taken, and the procedure was carried out as in the previous controls, always recording the crystallizer with the speaker and the tuner to analyze the results later.

3.1. Olloki samples (before passing through the city):

In Olloki, organic matter, sediments and stones (components for the controls) were collected at 10:30 hours. 11 minutes later, at 10:41 hours, river water was collected, and its temperature was measured using a submersible digital thermometer. As soon as the first bottle was filled, the countdown of 30 minutes began to determine the moment when the analysis had to start in the school laboratory (11:15 hours). Additionally, the time was measured to know when the next samples from the second point had to be collected using a second chronometer.

3.2. Samples at the Club Natación footbridges (as it passes through the city):

At 12:30 hours, samples of sediments, stones and organic matter were collected at the Club Natación footbridges, and once the chronometer reached 2 hours and 6 minutes, river water was collected (12:47 hours), and its temperature was measured. The countdown of 30 minutes started again to begin the experiment in the school laboratory at 13:20 hours.

3.3. Ororbia samples (after passing through the city):

Finally, at 16:35 hours, the components for the controls were collected, and 4 hours and 1 minute after collecting the samples at the Club Natación footbridges, the Ororbia samples were collected (16:49 hours), and the water temperature was measured. After 30 minutes, at 17:20 hours, the last experiment started in the school laboratory.

RESULTS

To obtain the results of the experiments, the previously recorded videos in the different controls and samples were analyzed. Through this analysis, the following results were obtained and summarized in Table 1 of the annex:

1. Negative control with distilled water:

During the distilled water control, two notes were found to be out of tune: G4 (2 cents) and E5 (10 cents). Additionally, two inconclusive results were obtained for C4 and C5. The rest of the notes remained in tune, as shown in Figure 4.

2. Positive controls:

2.1. Macroplastics control:

In the control with macroplastics, three variations in the frequency of the notes were measured: D4 (5 cents), E5 (3 cents), and G5 (8 cents) (Figure 5). The other notes remained in tune.

2.2. Microplastics control:

For the control with microplastics, four notes showed variations: D4 (5 cents), C5 (3 cents), D5 (3 cents), and G5 (12 cents) (Figure 6).

2.3. Control of the metal bottle for sample collection:

In this control, the notes E4 and E5 showed deviations of 2 cents, and C5 had a deviation of 5 cents. Additionally, an inconclusive result was recorded for D4, as seen in Figure 7.

2.4. Control of river salt concentration:

During the salt control, two inconclusive results were obtained for D4 and E5, and two notes showed deviations: G4 (5 cents) and G5 (2 cents) (Figure 8).

2.5. Control with organic matter:

In the Olloki control, three notes were found to be out of tune: C4, E4 (both with a 2-cent deviation) and D4 (9 cents). In the Club Natación control, three notes also showed variations: C4 and D5 (both with a 2-cent deviation) and G5 (10 cents) (Figure 9). Additionally, an inconclusive result was obtained for E5. In the Ororbia control, four notes were found to be out of tune: G4, C5 and D5 (all with a 2-cent deviation) and C4 (5 cents). Like the Club Natación control, an inconclusive result was obtained for E5.

2.6. Control with sediments:

Regarding the Olloki sediments control, three notes were found to be out of tune: C4, D4 and E5 (all with a 2-cent deviation). In the Club Natación control, two notes showed deviations: D4 (2 cents) and D5 (12 cents) (Figure 10). Additionally, an inconclusive result was recorded for E5. Finally, in the Ororbia control, three notes had a deviation of 2 cents for D4 and G5 and 5 cents for C4. Like the Club Natación control, an inconclusive result was obtained for E5.

2.7. Control with stones:

For the Olloki control, four notes with deviations were obtained: C4 (2 cents), D4 (5 cents), G4 (2 cents) and C5 (15 cents) (Figure 11). At the Club Natación, three out-of-tune notes were recorded: C4 and D4, both with a 2-cent deviation, and G4 with a 1-cent deviation. Additionally, an inconclusive result was obtained for E5. Finally, in Ororbia, three deviations were recorded: E4 (2 cents), E5 (2 cents) and G5 (5 cents).

3. River samples:

3.1. Olloki samples (before passing through the city):

In the first sample, only one note was found to be out of tune: the E5 (5 cents). In the second sample of Olloki, three notes were recorded with deviations, two with a deviation of 2 cents (D4 and C5) and one note with an 8-cent deviation (D5). However, for the third sample, the only note that experienced a change in its frequency was D4 (8 cents). In the fourth sample, there were two deviations in D4 (2 cents) and E5 (10 cents). Additionally, an inconclusive result was obtained for D5. During the fifth sample, several notes were found to be out of tune: C4 (5 cents), G4 (1 cent), and C5 (5 cents). However, as can be observed in Figure 12, the note that had the greatest deviation was D4 (10 cents).

3.2. Samples at the Club Natación footbridges (as it passes through the city):

In the first sample, no deviations were observed, but two inconclusive results were recorded for D5 and E5. In the second sample, only two notes were out of tune: C4 (2 cents) and D4 (10 cents) (Figure 13). Like the previous sample, an inconclusive result was obtained for E5. In the third sample, three notes were out of tune: D4, G4 and E5 (all with a 2-cent deviation). For the fourth sample, three notes also showed deviations: D4 (8 cents), G4 (1 cent) and E5 (2 cents). Finally, in the fifth sample, only two notes with variations were recorded: C5 (2 cents) and G5 (5 cents).

3.3. Ororbia samples (after passing through the city):

In the first sample, four notes were out of tune: G4 (2 cents), C5 (10 cents) (Figure 14), D5 (5 cents) and G5 (7 cents). Additionally, an inconclusive result was obtained for E5. During the analysis of the second sample, five deviations were obtained: 1 cent (C4), 10 cents (D4), 2 cents (E4), 5 cents (G4) and 7 cents (G5). Like the first sample, an inconclusive result was obtained for E5. In the third sample, two deviations were obtained in D4 (1 cent) and D5 (5 cents). Additionally, an inconclusive result was obtained for E5. The fourth sample only presented deviations in D4 (10 cents) and E4 (5 cents). An inconclusive result was obtained for E5 again. Finally, in the fifth sample, deviations of 2 cents were obtained for C4 and G5, and a deviation of 10 cents was obtained for E5 (Figure 15). There were also three inconclusive results for D4, E4 and D5.

DISCUSSION

The notes C4 and C5, the same note in different scales, have shown inconclusive results in the distilled water control. Therefore, without the data from the negative control, the results in the positive controls and samples cannot be reliable.

On the other hand, it can be noted that E5 is not a reliable musical note for obtaining results, as more than one-third of its measurements were inconclusive. The same applies to D4, as the values obtained in the control with metal bottles and the river's salt concentration were inconclusive.

Finally, the results of the notes E4 and G4 cannot be analyzed or considered since the positive controls for macroplastics and microplastics did not show any deviation.

Given the number of inconclusive results and the variables provided by all the notes, it was decided to create a comparative graph (Figure 16) for note G5. This is because it is the only note that has shown no inconclusive results and has also not shown deviations in the distilled water control, but it has shown them (to a greater extent compared to the other notes) in the controls with macroplastics and microplastics.

CONCLUSIONS

After analysing the results of note G5, it can be concluded that sound can be a good method for the detection of microplastics in water, as observed in the controls for microplastics and macroplastics. Additionally, the samples and their controls after the passage of water through the city also indicate the presence of something in the water. Considering that Pamplona is the first major city along the course of the Arga River, it is also the first significant pollution source. By subtracting the values from the controls in Ororbia from the samples themselves and the controls for macroplastics and microplastics, deviations can still be detected, which may indicate the presence of these particles in the water.

RECOMMENDATIONS

During this research, there were several issues with the speakers used, as the model used, being IPX7-rated, can withstand splashes but not prolonged immersion in water. Therefore, it would be better to conduct future experiments using an IPX8-rated speaker that is specifically designed for underwater use. However, it should be noted that those speakers tend to be larger and more expensive, requiring a recalculation of the necessary water volume for the experiments.

Furthermore, the G5 samples from the positive control with sediments from Ororbia contained small stones. As a result, it is unclear whether the deviation shown in that control (2 cents) is truly due to the sediments or the presence of the stones, which had a higher deviation value in the stone control at the same location (5 cents). By using improved sampling equipment such as waterproof pants and boots, better sediment control samples could be obtained.

Lastly, an important future improvement for this research project could be to investigate whether the variations caused by microplastics affect the communication of aquatic fauna. To conduct this study, it would be necessary to establish the equivalence between KHz (the frequency scale used by animals) and cents (the scale measured by the tuners) as a first step in understanding the impact on animal communication.

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ANNEX OF FIGURES

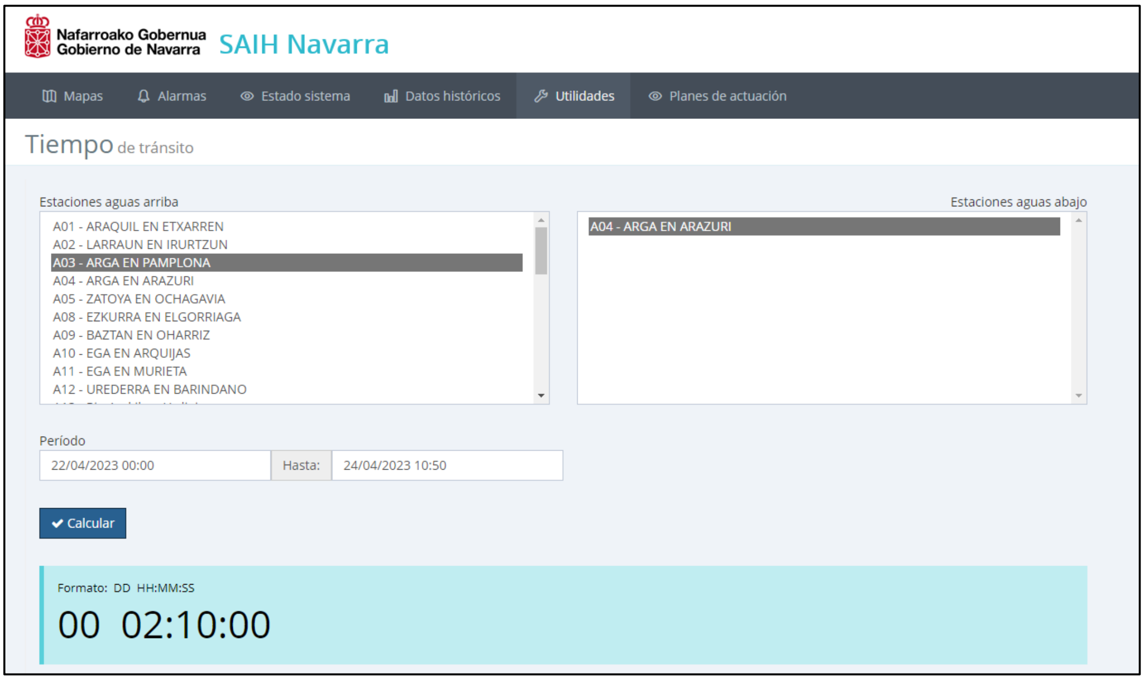

Figure 1. Transit time of water in the Arga River between the two stations in the city of Pamplona, between April 22nd and April 24th, 2023. Source: SAIH Navarra, Government of Navarra, 2023.

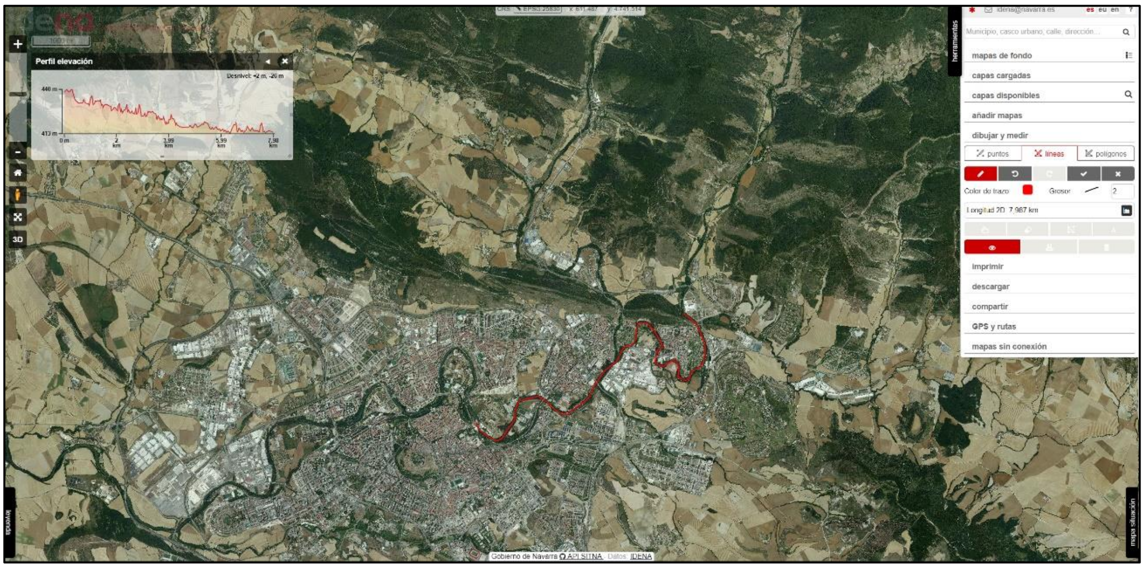

Figure 2. Calculation of the distance between the sampling site in Olloki and the Club Natación footbridges. Source: Own elaboration based on the IDENA web application of the Government of Navarra.

Figure 3. Calculation of the distance between the sampling site in the Club Natación footbridges and Ororbia. Source: Own elaboration based on the IDENA web application of the Government of Navarra.

Sample/Note	C4	D4	E4	G4	C5	D5	E5	G5
Distilled Water	x	0	0	2	x	0	10	0
Macroplastics Control	0	5	0	0	0	0	3	8
Microplastics Control	0	5	0	0	3	3	0	12
Metal bottle	0	x	2	0	5	0	2	0
Salinity control	0	x	0	5	0	0	x	2
Organic Matter Control Olloki	2	9	2	0	0	0	0	0
Sediment Control Olloki	2	2	0	0	0	0	2	0
Stones Control Olloki	2	5	0	2	15	0	0	0
Olloki sample 1	0	0	0	0	0	0	5	0
Olloki sample 2	0	2	0	0	2	8	0	0
Olloki sample 3	0	8	0	0	0	0	0	0
Olloki sample 4	0	2	0	0	0	x	10	0
Olloki sample 5	5	10	0	1	5	0	0	0
Organic Matter Control Club Natación	2	0	0	0	0	2	x	10
Sediment Control Club Natación	0	2	0	0	0	12	x	0
Stones Control Club Natación	2	2	0	1	0	0	x	0
Club Natación sample 1	0	0	0	0	0	x	x	0
Club Natación sample 2	2	10	0	0	0	0	x	0
Club Natación sample 3	0	2	0	2	0	0	2	0
Club Natación sample 4	0	8	0	1	0	0	2	0
Club Natación sample 5	0	0	0	0	2	0	0	5
Organic Matter Control Ororbia	5	0	0	2	2	2	x	0
Sediment Control Ororbia	5	2	0	0	0	0	x	2
Stones Control Ororbia	0	0	2	0	0	0	2	5
Ororbia sample 1	0	0	0	2	10	5	x	7

Ororbia sample 2	1	10	2	5	0	0	x	7
Ororbia sample 3	0	1	0	0	0	5	x	0
Ororbia sample 4	0	10	5	0	0	0	x	0
Ororbia sample 5	2	x	x	0	0	x	10	2

Table 1. Frequencies of the notes played in the experiment and variation in cents, for the negative, positive controls and the samples from Olloki, Pamplona and Ororbia. Source: Own elaboration.

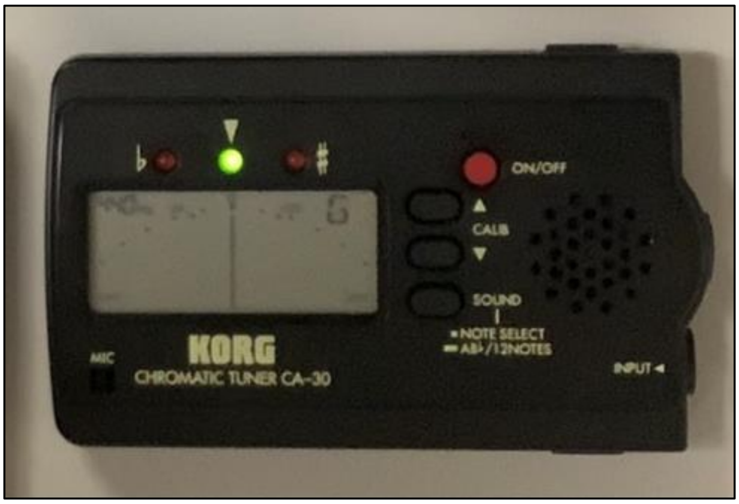

Figure 4. Tuner displaying the frequency value and its lack of variation in the measurement of the negative control with distilled water. Source: Own elaboration.

Figure 5. Tuner displaying the frequency value and its variation in the measurement of the positive control with macroplastics. Source: Own elaboration.

Figure 6. Tuner displaying the frequency value and its variation in the measurement of the positive control with microplastics. Source: Own elaboration.

Figure 7. Tuner displaying the frequency value and its variation in the measurement of the positive control with the metal bottle. Source: Own elaboration.

Figure 8. Tuner displaying the frequency value and its variation in the measurement of the positive control with the salinity concentration. Source: Own elaboration.

Figure 9. Tuner displaying the frequency value and its variation in the measurement of the positive control with organic matter from Club Natación footbridges. Source: Own elaboration.

Figure 10. Tuner displaying the frequency value and its variation in the measurement of the positive control with sediments from Club Natación. Source: Own elaboration.

Figure 11. Tuner displaying the frequency value and its variation in the measurement of the positive control with stones from Olloki. Source: Own elaboration.

Figure 12. Tuner displaying the frequency value and its variation in the measurement of the sample from Olloki. Source: Own elaboration.

Figure 13. Tuner displaying the frequency value and its variation in the measurement of the sample from Club Natación. Source: Own elaboration.

Figure 14. Tuner displaying the frequency value and its variation in the measurement of the sample from Ororbia. Source: Own elaboration.

Figure 15. Tuner displaying the frequency value and its variation in the measurement of the sample from Ororbia. Source: Own elaboration.

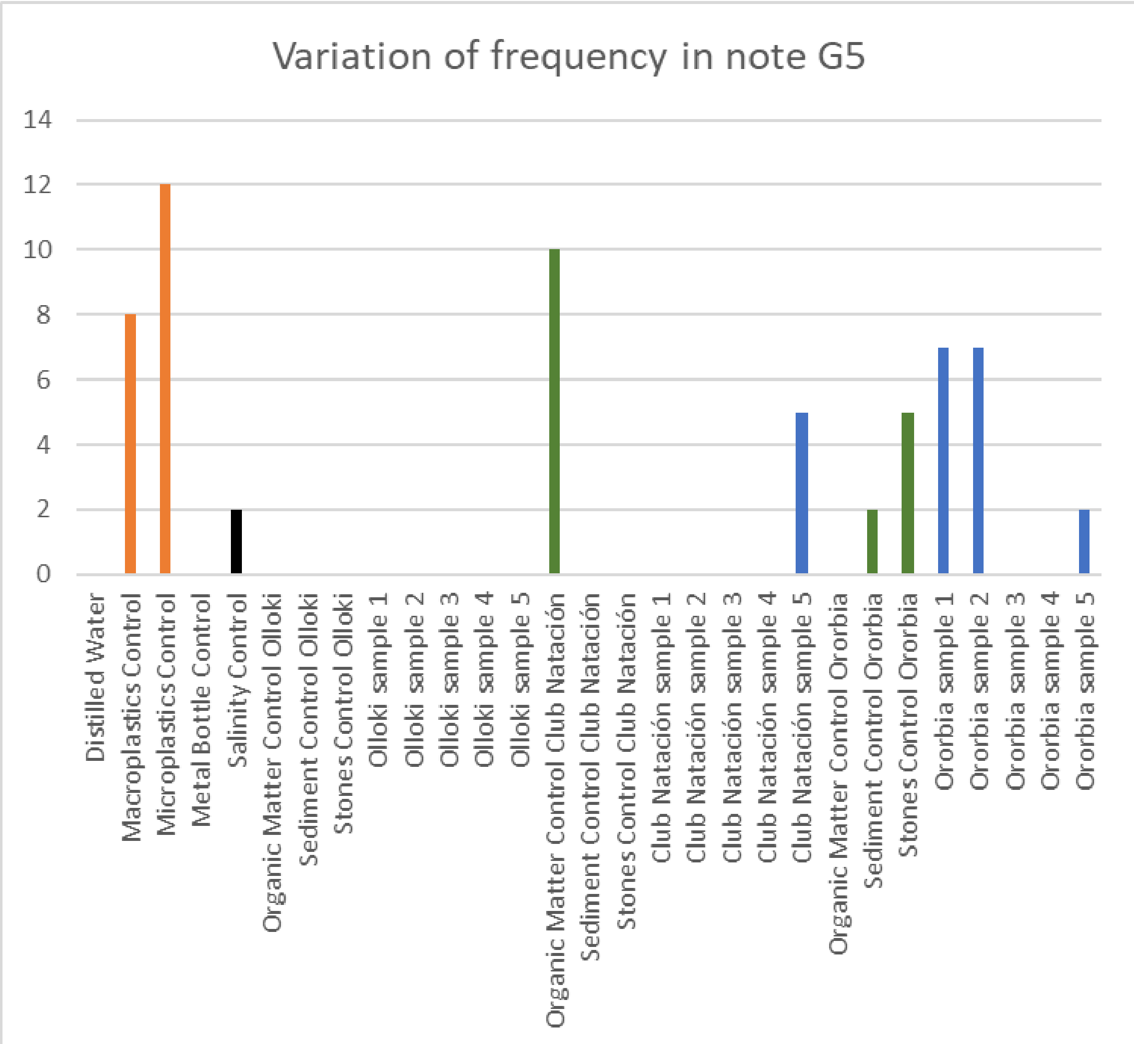

Figure 16. Variation of frequency in the different samples and controls in note C5. Source: Own elaboration.