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**SECONDARY
SCHOOL STUDENTS'
MISCONCEPTIONS
ABOUT
MICROORGANISMS.
A LABORATORY
SEQUENCE TO
LEARN ABOUT THEIR
PRESENCE AND
FUNCTION**

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Abstract: Microorganisms are central to life. However, students have several misconceptions and think of microorganisms primarily as disease-causing hazardous germs, ignoring all the crucial roles they play as well as the benefits we obtain from them. All these misconceptions can significantly hinder the education process, both for students and for teachers. Therefore, the aim of this study was to identify secondary school students' misconceptions about microorganisms, and to design, implement and evaluate a laboratory sequence that would promote the understanding of concepts as well as students' critical thinking about the presence and relevance of microorganisms. Students were asked to complete a questionnaire that consisted of open questions, drawings and true/false questions. They also performed three laboratory experiments related to the ubiquity of microorganisms and the antimicrobial effect of different biocides. Our results uncovered many misconceptions related to the presence and functions of microorganisms. However, the didactic sequence proposed in this study enabled students to successfully understand diverse concepts related to microorganisms and promoted students' critical thinking.

Keywords Misconceptions · Education · Didactic sequence · Microbe · Questionnaire.

INTRODUCTION

Microorganisms predominate worldwide, comprising the most diverse and widespread group in the Biosphere (Atlas and Bartra 1986). The species richness and biomass of microorganisms exceed that of all plants and animals on Earth (Pedrinaci et al. 2013) and they are highly ubiquitous, being able to live in very diverse environments (Anitori 2012). They play crucial roles in a number of processes that ensure the continuation of life on Earth, significantly contributing to biogeochemical cycles of fundamental

elements, as well as to the physiology of plants and animals (Casamayor and Gasol 2012). Additionally, they are employed in relevant industries connected with foods (e.g. baking, dairy products processing, brewing), medicines, sewage treatment and biofuels (Waites et al. 2001).

The importance of microorganisms is well reflected in the international scientific endeavor. However, scientific learning does not go in parallel with research. Microbiology is not studied in depth during primary and secondary education, even less the role of microorganisms in ecosystem balance and in our everyday life (Marbach-Ad et al. 2009; Merkel 2012). Moreover, students have their own pre-instructional notions or misconceptions, which are formed based on their own observations, on information gained from diverse sources in their environment (e.g. written and electronic media, family, friends), and on their own interpretation of all these information (Allen 2014; Vosniadou 2009). They are constituted by cognitive, affective and symbolic contents and often do not coincide with the knowledge developed by the scientific community (Furió et al. 2006).

In particular, most primary and secondary school students continue to think of microorganisms as disease-causing hazardous germs and have very little idea of their actual relevance (Jones and Rua 2006; Karadon and Sahin 2010). Other misconceptions include that microbes are nonliving things, that bacteria, viruses and unicellular fungi are all the same, and that microorganisms are only found in unhygienic conditions (Byrne et al. 2009). Concerning the ecosystem balance, many students are not aware that microorganisms decompose dead organic matter and believe plants directly assimilate nutrients from dead animals, neglecting the crucial role of microorganisms in nutrient and matter cycling, and even ignoring the

relationship between decomposition and nutrient cycling (Allen 2014; Leach et al. 1996). Some others also refuse the beneficial technological applications of microorganisms (Byrne et al. 2009).

All these pre-instructional misconceptions can significantly hinder the education process (Kose 2008). They constitute the points on which all further knowledge is based, and are often difficult to change (Ausubel 1968; Duit and Treagust 2003). They provide the interpretation schemes to which students fit lessons and, thus, misconceptions cannot simply be replaced with correct technical knowledge; rather, they need to be mentally re-organised and adapted to the scientific theories (Reinfried et al. 2012). Accordingly, various studies showed that lessons incorporating everyday notions are more successful (Reinfried et al. 2010; Wiesner 1995). Therefore, developing appropriate tools for identifying student's ideas and designing didactic sequences that promote student-centered approaches that focus on critical thinking skills and emphasize active learning (Handelsman et al. 2004) has been a major challenge in science teaching over the past few years (Merkel 2012; Waldrop 2015).

This study has two main objectives:

- (1) to identify secondary school students' misconceptions about microorganisms.
- (2) to design, implement and evaluate a laboratory sequence to promote students' critical thinking about the presence and relevance of microorganisms both in ecosystems and in our everyday life.

METHODS

SAMPLE

The sample of this study was comprised of 18 students enrolled in a secondary school located in San Sebastian, a city with more than 180,000 inhabitants (Basque Country, Spain). They were all second grade

students: given that the Basque Government curriculum establishes that microbiology must be taught during the third grade, the second grade was the best stage to identify conceptions of students acquired outside the school. The age of the participants averaged 14.7, and there were 7 girls and 11 boys, but gender was not considered an important factor in this study.

TASKS

Students were asked to complete two main tasks, during April 2015. The first task consisted of a questionnaire that included three open questions about basic concepts related to microorganisms as well as diverse true/false questions, and students were also requested to draw a microorganism. Open questions included:

- (1) What are microorganisms? Are they alive?
- (2) Are microorganisms visible to the naked eye? How can they be seen?
- (3) Are microorganisms good or bad? Why?

True/false questions included:

- (1) There are a lot of microorganism types
- (2) Microorganisms are only found in unhygienic conditions
- (3) Everything we eat and drink is free of microorganisms
- (4) We have microorganisms throughout the body
- (5) Microorganisms are also found in volcanoes and deserts
- (6) The unique function of microorganism is to decompose organic matter
- (7) Our lives depend on many microorganisms
- (8) Microorganisms are essential to make bread, yogurt and cheese
- (9) Microorganisms are fundamental for life
- (10) Some microorganisms protect us

- (11) All microorganisms are germs
(12) When we get sick we take antibiotics to kill harmful microorganisms

The other task consisted of activities to promote the understanding of concepts as well as students' critical thinking about microorganisms. It included three experimental activities that were performed in the laboratory. The aim of the first experiment was to make students think critically about the presence and ubiquity of microorganisms in our surroundings. Following Gamazo et al. (2013), students were provided with LB or PDA agar plates where they inoculated microorganisms found in leaf litter, soil, a puddle, a yogurt, their mouth and their fingers. The other two experiments were related to the antimicrobial effect of different biocides: one aimed to demonstrate the effects of natural biocides such as those substances found in garlic (Grainger and Hurst 2008; López Pérez 2011), whereas the other analysed the effect of commercial antibiotics (Lopez Pérez and Boronat Gil 2011). LB agar plates previously colonized by *Escherichia coli* were incubated with fresh and boiled garlic, as well as with three dilutions of ampicillin (1/10, 1/100, 1/1000). A control without ampicillin was also used. All the plates were incubated at 37 °C for 48 h. After the incubation students analysed the plates, described their observations and answered questions about the presence and appearance of microorganisms. These responses were compared to the ones obtained in the questionnaire and used to evaluate the success of the didactic sequence.

DATA ANALYSIS

Answers to the open questions were coded within the categories that emerged from examining and re-examining the results, following Byrne (2011; Table 1). Qualitative analysis of drawings was also undertaken by attributing characteristics of the drawings

to particular categories related to their appearance and their structural complexity (Byrne 2011; Table 2). When students did multiple different drawings, each was analysed independently. Quantitative tallying of the different categories was undertaken to provide the percentage of students for each category, for both open questions and drawings. In the case of drawings, the frequency of each category was provided together with the percentage of students, due to multiple drawings. Answers to the true/false questions were expressed as percentage. Results obtained after completing the three tasks were compared to the ones obtained at the beginning of the study in order to assess students' progress and, thereby, the success of the activities proposed in this study.

RESULTS

OPEN QUESTIONS

The vast majority (83.3%) responded that microorganisms are living beings (Table 3). Indeed, 66.7% of the students defined microorganisms as very small or microscopic living organisms. However, open question 1 revealed several misconceptions: some students defined microorganisms as bacteria, ignoring many other groups of microorganisms, and the category 'other' included answers such as 'microorganisms are composed of decomposed organisms', 'microorganisms are very small particles' and 'microorganisms are microscopic organs'. Only 33.3% of the students mentioned some of the functions performed by microorganisms, including decomposition of dead organic matter, reproduction and the fact that they eat other microorganisms.

Regarding open question 2, there was a consensus view that microorganisms cannot be seen with the naked eye because they are too small, and all but one stated that a microscope is required to see microorganisms.

Few students answered that

microorganisms are all beneficial (5.6%) or all harmful (22.2%), the most common response to open question 3 being: 'some microorganisms are good and some others are bad' (Table 3). 16.7% of the student did not justify their answer. The most common (66.7%) argument was that microorganisms cause and/or transmit diseases and infections, and was used to support that microorganisms are harmful. Some students even mentioned death, cancer and amputation due to microorganism infection or stated that 'microorganisms are disease'. The reasons used to argue that microorganisms are beneficial included the role of microorganism in decomposition (16.7%) and supporting life (11.1%), but also some misconceptions such as 'good microorganisms heal wounds', 'good microorganisms help the body fight disease', and 'cells are good, viruses bad'.

DRAWINGS

One student left the space blank. Half of the students did either a single drawing, or several but very similar drawings. The rest did multiple drawings that were coded within different categories. In total, 33 drawings were analysed. Only two were classified as 'abstract/other' and no one drew multi-cellular organisms, the rest 31 drawings representing single cells (Fig. 1; Table 4). Bacterial cells were the most common: 61.1% of the students did at least one bacterial cell, 16 in total. Almost half of the students (44.4%) drew an amorphous/amoeboid cell, and 33.3% of the students attributed the features of typical eukaryotic cells to 6 of the drawings.

Concerning the structural complexity of drawings, the 4 categories were more equitably represented (Table 4). Most of the students (55.5%) did partial drawings, distinguishing only extracellular components (mainly cilia; 6 drawings, 22.2% of the students) or only cytosolic components (mainly vacuole-like

organelles; 8 drawings, 33.3% of the students). However, many students drew only the outline of microorganisms (9 drawings, 38.9% of the students), or, in contrast, demonstrated to have a more complete/complex image of microorganism (8 drawings, 44.4% of the students).

TRUE/FALSE QUESTIONS

Students answered correctly between 5 and 11 true/false questions, 9 on average. Concerning the percentage of students that answered correctly each question, it ranged between 43.7% and 93.7% (Table 5). More specifically, the question related to the presence of microorganisms in volcanoes and deserts (question 5) was the one that students failed the most, whereas questions related to the diversity of microorganisms, their presence in our body and their role in supporting life were answered correctly by the vast majority (questions 1, 4 and 9). In general, more students answered correctly the questions related to the ubiquity of microorganisms (questions 1 to 4), than the ones related to their functions and roles (questions 6 to 11).

LABORATORY EXPERIMENTS

Very diverse microorganism colonies grew in the agar plates (Fig. 2). Although the majority answered correctly most true/false questions and seemed to be aware of the ubiquity of microorganisms, many students expressed surprise when observing all these colonies. Some were reticent to believe that they have microorganisms like the ones grown in the plates in their mouths and fingers: 'It seems my hands are clean, I cannot have all these microorganisms in my fingers'. One student even believed water in puddles is drinkable because it does not contain microorganisms. Another was surprised to see that 'some microorganisms are found both in fingers and in the mouth'. Besides,

many students did not understand the role of microorganisms in all these environments and were worried about the potential harm they could cause: 'What are these microorganisms doing in a leaf?'; 'Do plants get sick when they have these microorganisms?'; 'Would we get sick if we ate these materials?'

Concerning the size and appearance of microorganisms, there was a consensus view that microorganisms cannot be seen with the naked eye. This experiment corroborated their conception because diverse microorganisms grew from apparently microorganism-free inoculums, but also demonstrated that microorganisms can create visible colonies of different color, size and form.

E. coli around the fresh garlic disappeared (Fig. 3a), indicating fresh garlic contains natural biocide substances that can kill microorganisms, at least this species. In contrast, the boiled clove did not affect *E. coli*, indicating these substances are deteriorated when boiling the garlic. Similarly to fresh garlic, ampicillin also killed *E. coli* (Fig. 3b), but the effect greatly depended on its concentration: the higher the concentration, the bigger the effect. Generally, all students understood the antimicrobial effect of both biocides, but some were concerned about the potential consequences of eating fresh garlic and taking antibiotics for our stomach microbiota: 'Would microorganisms in our stomach die if we ate garlic?'; 'Do antibiotics kill only harmful microorganisms?'. Some others even suggested substituting fresh garlic for antibiotics.

Overall, despite the good results obtained in the true/false test, comments arisen during the three experiments revealed that students had several misconceptions related to the presence and functions of microorganisms. Results obtained in the three experiments uncovered many of these perceptions and helped to accurately explain how ubiquitous

microorganisms are and how important they are in all these environments, and promoted the critical thinking of students.

DISCUSSION

This study revealed that secondary school students have multiple misconceptions about microorganisms. Some of these misconceptions have been thoroughly reported in the literature for a relatively long time (Allen 2014; Byrne 2003; Sequeira and Freitas 1986), suggesting that misconceptions about microorganisms have not evolved. In particular, some students do not clearly distinguish particles, inorganic and organic matter and living beings (Byrne 2003) and, thus, have difficulties to tell whether microorganisms are living beings or not (Allen 2014), which leads to many other misconceptions related to their functions. For instance, many students believe that primary producers take nutrients directly from dead organic matter, neglecting the whole decomposition process carried out by microorganisms and other decomposers (Byrne et al. 2009). These findings agree with our results, since only 16.7% of the students mentioned the decomposing role of microorganisms.

In contrast, all the students seemed to know that microorganisms are too small to be seen without magnification, which agrees with Simonneaux (2000) and Vasquez (1985). However, these studies also highlighted that while there is a good understanding that microorganisms are very small and the term 'microscopic' is used widely, there is little real understanding of what this means with respect to the actual size of microorganisms.

Moreover, the majority stated that microorganisms are disease- or infection-causing organisms. The pathogenic view of microorganisms, which Raichvarg (1995) referred to as 'microbe-mania', is developed

from an anthropogenic perspective and is deep-rooted worldwide (Byrne and Sharp 2006; Springer and Ruckel 1992). Nonetheless, the mechanisms of infection and subsequent recovery are poorly understood (Jones and Rua 2006; Kalish 1996), and some students even attributed non-microbial disease to a microbial cause (e.g. cancer; also reported by Byrne [2011]). Besides, it must be noted that no one mentioned the use of microorganisms in industries. These results agree with previous research showing that the use of microorganisms to manufacture food and medical products or for environmental benefit is poorly understood (Simonneaux 2000; Williams and Gillen 1991).

In contrast to other studies that reported animal-like, anthropomorphized and multiple-cell representations (Byrne and Sharp 2006; Byrne 2011), drawings in this study represented single cells. Compared to Byrne and Sharp (2006), drawings in this study had more details of internal features and specific cellular structures. Nevertheless, our results suggest that students ignore the presence of colonial and multicellular microscopic living organisms (e.g. protists, molds, rotifers), or at least do not consider they are microorganisms. Additionally, although bacterial cells were the most common, many students attributed the features of typical eukaryotic cells to their drawings, which, together with the answer some students gave to the open question 3 ('cells are good, viruses bad'), casts doubt on whether students distinguish cells as the unit of life, and unicellular eukaryotic organisms.

In general, the vast majority answered correctly all the true/false questions. Despite these successful results, comments arisen during the laboratory experiments revealed several misconceptions related to the presence and functions of microorganisms, suggesting that not all the tools are equally effective to detect misconceptions. Although

interviews and diagnostic test acquired strong support (Peterson et al. 1986; Treagust 1988), these methods allow students to repeat what they learned in class without revealing their misconceptions (Scherz and Oren 2006). In the case of true/false questions, it is relatively easy to answer correctly by mere intuition, and, at worst, the probability of guessing by chance is 50%. In contrast, drawings reveal students' true understanding of basic scientific concepts, providing a holistic perspective of their inner world that includes their thoughts, notions and feelings (Kose 2008; White and Gunstone 1992). This study showed that laboratory experiments are also highly efficient to identify misconceptions, because they offer a more relaxed environment in which students express their thoughts and doubts.

Additionally, recent research reported that laboratory didactic sequences foster student motivation. They involve the knowledge and development of procedures, as well as the use of tools required for investigation (Osborne and Dillon 2010), and they promote learning by inquiry (Rissing and Cogan 2009; Wolf and Fraser 2008), thus being highly successful for teaching science (Lock 2010). The first laboratory exercise was very convenient to show that microorganisms live almost everywhere and it helped explain that microorganisms are crucial in all these environments, not necessarily disease-causing dangerous organism. It also led to mention why we cannot eat or drink from anywhere, and why it is important to wash our hands and teeth. The other laboratory exercises were useful to show that there are defense mechanisms against potentially harmful microorganisms. The last activity also revealed the importance of the antibiotic dosage, a topic of increasing concern due to the widespread imprudent use of antibiotics (Lecky et al. 2010).

Overall, despite their relevance, this study revealed that students have several misconceptions about microorganisms, especially about the functions they perform. It is crucial to identify these misconceptions and design didactic sequences that guarantee students will experiment and meditate about microorganisms and their presence, functions and relevance in Earth.

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Living	Q1		Q2		Q3	
	Definition/function ^a	Visible	Tool	Good/bad	Reason ^{ab}	
Yes	Very small/microscopic organisms/cells	Yes	Microscope	Good	Disease/infection-causing and/or transmission (-)	
No	Bacteria	No		Bad	Decomposition (+)	
	Other			Both/depend on the type	Eat other microorganism (+)	
	Decomposition				Heal wounds/fight diseases (+)	
	Reproduction				Support life (+)	
	Eat other microorganisms/bacteria					

^a Categories within the 'Definition/function' and 'Reason' columns are not exclusive.

^b Positive (+) and negative (-) signs in the 'Reason' column indicate whether the argument was used to state that microorganisms are good (+) or bad (-).

Table 1 Categories used to classify responses to the three open questions.

Appearance/morphology	Structural complexity
Amorphous/amoeboid	Simple, only outline
Bacterial cell	Extracellular components (e.g. cilia/flagella)
Eukaryotic cell	Cytosolic components (e.g. nucleus, vacuoles)
Abstract/other	Complex, extracellular and cytosolic components

All categories are exclusive.

Table 2 Categories used to classify the appearance/morphology and the structural complexity of the drawings.

Living	Q1		Q2				Q3				
	%	Definition/function	% ^a	Visible	%	Tool	% ^b	Good/bad	% ^b	Reason	% ^a
Yes	83.3	Very small/microscopic organisms/cells	66.7	Yes	0	Microscope	94.4	Good	5.6	Disease/infection-causing and/or transmission (-)	66.7
No	16.7	Bacteria	11.1	No	100			Bad	22.2	Decomposition (+)	16.7
		Other	16.7					Both/depend on the type	66.7	Eat other microorganism (+)	11.1
		Decomposition	11.1							Heal wounds/fight diseases (+)	11.1
		Reproduction	11.1							Support life (+)	11.1
		Eat other microorganisms/bacteria	11.1								

^a Total is more than 100% because categories are not exclusive.

^b Total is less than 100% because some students did not answer the questions.

Table 3 Percentage of students (%) for each category defined for open questions.

Appearance/morphology	Frequency % ^a	Structural complexity	Frequency % ^a
Amorphous/amoeboid	9 44.4	Simple, only outline	9 38.9
Bacterial cell	16 61.1	Extracellular components (e.g. cilia/flagella)	6 22.2
Eukaryotic cell	6 33.3	Cytosolic components (e.g. nucleus, vacuoles)	8 33.3
Abstract/other	2 11.1	Complex, extracellular and cytosolic components	8 44.4

^aTotal is more than 100% due to multiple drawings.

Table 4 Frequency of drawings and percentage of students (%) for each category defined for drawings.

	Question	True ^{ab}	False ^{ab}
1	There are a lot of microorganism types	93.7	6.3
2	Microorganisms are only found in unhygienic conditions	12.5	87.5
3	Everything we eat and drink is free of microorganisms	18.7	75.0
4	We have microorganisms throughout the body	93.7	6.2
5	Microorganisms are also found in volcanoes and deserts	43.7	43.7
6	The unique function of microorganism is to decompose organic matter	37.5	62.5
7	Our lives depend on many microorganisms	75.0	18.7
8	Microorganisms are essential to make bread, yogurt and cheese	81.3	18.7
9	Microorganisms are fundamental for life	93.7	0
10	Some microorganisms protect us	81.2	6.2
11	All microorganisms are germs	12.5	68.7
12	When we get sick we take antibiotics to kill harmful microorganisms	50.0	31.2

^a Bold characters indicate the correct answer.

^bTotal may be less than 100% due to blank or null answers.

Table 5 Results from the true/false questions.

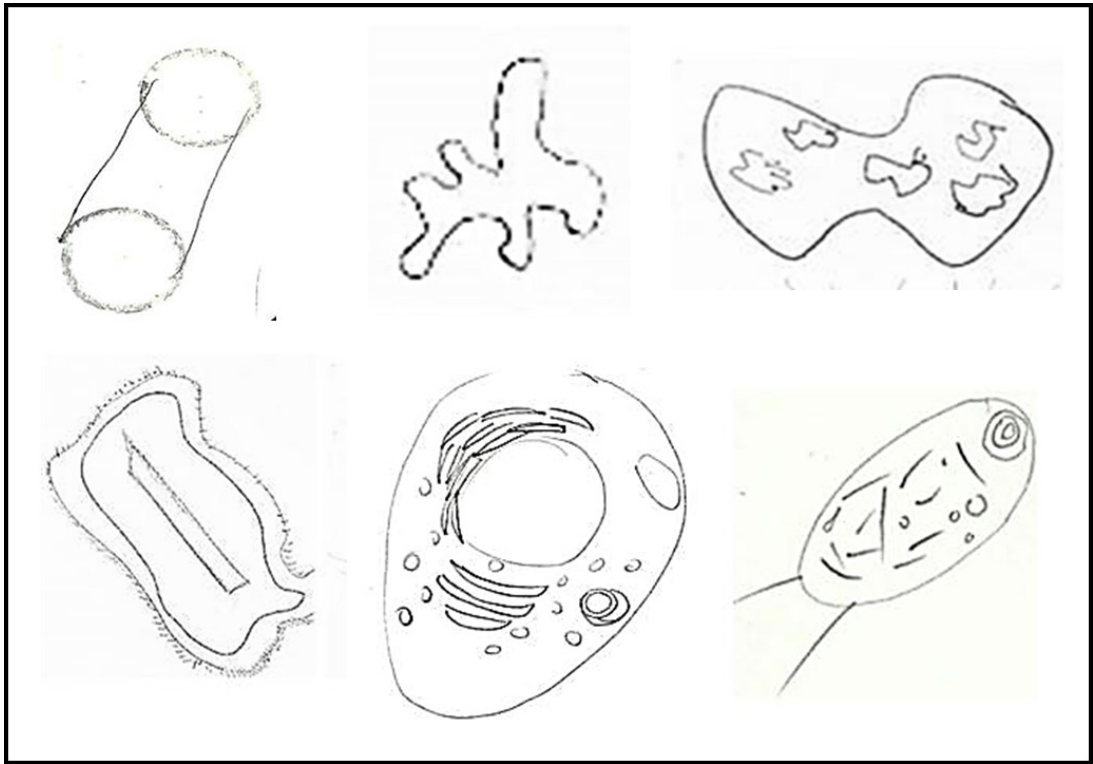


Fig. 1 Examples of drawings.



Fig. 2 Pictures of microorganism colonies grown in Experiment 1.

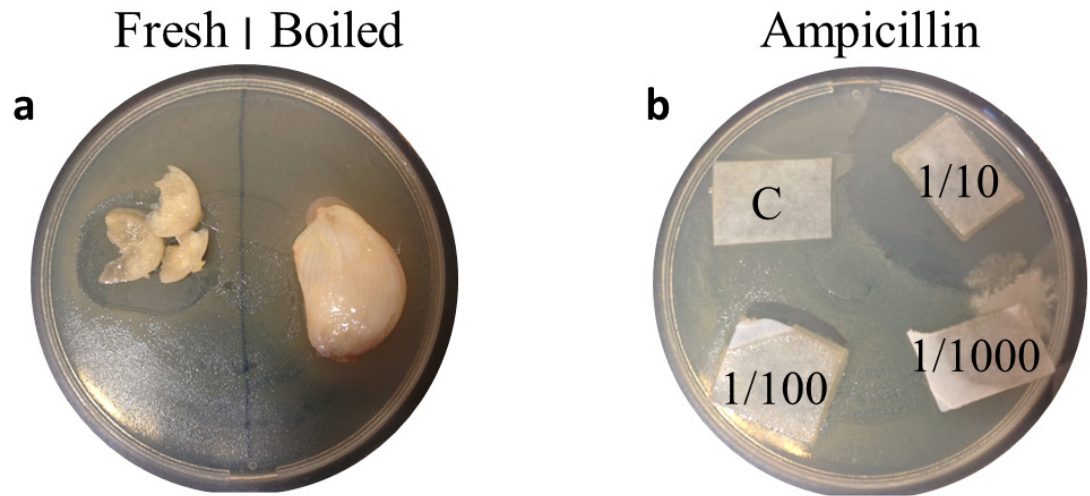


Fig. 3 Results obtained in the two experiments related to the antimicrobial effect of different biocides. (a) Effect of natural substances produced by garlic: fresh (left) and boiled (right). (b) Effect of three dilutions of ampicillin. C refers to the control without antibiotics