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Research Article

Pollution Abatement of Heavy Metals in Different Conditions by Water Kefir Grains as a Protective Tool against Toxicity

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This research paper addresses the hypothesis that Water Kefir grains can be used as absorbers of metal ions and reports the first application of the Water Kefir grains as a protective tool against toxicity by heavy metal ions. The aim of this study is to evaluate the concentration of heavy metal ions in several Water Kefir solutions during the fermentation process under various conditions. Two colonies of Water Kefir grain were used, and the concentrations of Cd, Co, Cr, Cu, Mn, Ni, Pb, Ba, and Ca were measured in Water Kefir grain solutions at different contact times (0, 24, 48, and 72 hours), different pH values in citric and acetic buffers, and different Water Kefir grains/metal solution ratios, with and without sucrose (5%). Optical emission spectroscopy was used to measure the concentrations of metal ions. Among the tested experimental conditions, the best combination for pollution abatement is sucrose (5%), contact time 24 hours, starting $pH = 4.5$, acetate buffer, and Kefir grains/metal solution ratio 1 : 1. In these conditions, the heavy metal abatement by Water Kefir grains is particularly effective for Cr and Pb (70%) and good for Cu, Ni, and Mn (50%).

1. Introduction

Water kefir is an acid, softly alcoholic and fragrant fermented drink whose fermentation is started with solid Water Kefir grains. These Water Kefir grains contain an insoluble polysaccharide and bacteria and yeasts responsible for the fermentation [1–3]. The insoluble Water Kefir grains act as inoculum when added to a mixture of water and sugar (sucrose), possibly with extra ingredients such as lemon, dried figs, and many others. After 24–48 hours of incubation, a yellowish fermented drink is obtained; it has a fruity aroma and an acidic, slightly sweet, and slightly alcoholic taste [2, 4–9].

Water Kefir is accessible worldwide, but the real origin of the grains is still uncertain. It has been proposed that the Water Kefir grains came from the *Opuntia* cactus. “Water kefir” is the typical name in Western Europe, but also other appellations are in use for this fermented beverage, depending on the country, such as “African bees,” “California bees,” “Japanese beer seeds,” “ginger beer plants,” “Tibicos,” “Tibi grains,” “ale nuts,” “balm of Gilead,” “Bèbées,” and “sugary kefir grains” [10, 11]. In general, Water Kefir beverage is used as a dietary supplement to rebalance the intestinal microflora and as a probiotic supplement [12–20].

Nowadays, investigations on Water Kefir grains are still very incomplete, and most of the scientific research available has analysed its biological diversity [1, 10, 21]. The structure and the biochemical composition of the Water Kefir grain polysaccharide has been also studied [22–24]. The microbial diversity of Water Kefir is based on a constant consortium of principally lactic and acetic acid bacteria and yeasts; however, different Water Kefir colonies display different microbial species [25, 26]. Nevertheless, the fermentation conditions, pH modification, and presence and concentration of heavy metals have been poorly reported compared to the vast investigation of the microbial diversity of Water Kefir [3, 27–30].

Water Kefir colonies could interact with heavy metals both physically and chemically due to their structure and functional groups.

In general, the chance of heavy metal contamination in food and water is high due to the increasing anthropic activities. For these reasons, it is important to define/

establish the chemical quality of water, particularly the content of heavy metals, in order to evaluate the possible human health risk [31–37]. Metals such as zinc, copper, iron, and manganese are essential since they play an important role in biological systems, whereas chromium, lead, and cadmium are toxic even in traces. The essential metals can also produce poisonous effects when the intake is excessively elevated. As a consequence, there are concentration limits of them that are established for food and water in most countries.

Therefore, the focus of this work is to understand if Water Kefir grains can be used for pollution abatement of heavy metal ions at different conditions (contact time, starting pH, buffer type, and metal concentration). Moreover, the study deeps the importance of the fermentation process (in sucrose presence) for the adsorption of metal ions and so identifying the best condition for possible application of Water Kefir grains as a protective tool against toxicity. To this end, two Water Kefir grains were tested, and the concentrations of heavy metals were evaluated in a Water Kefir fermentation process as a function of time (24, 48, and 72 h) in the presence or absence of sucrose at different starting pH values (pH = 3.5, 4.5, and 6.0) in different buffer types (acetate and citrate). Kefir/metal solution ratios of 1 : 1 and 1 : 10 were also evaluated. An analytical method originally used to analyse metals in natural water was adapted for the determination of Cd, Co, Cr, Cu, Mn, Ni, Pb, Ba, Ca, K, Mg, and Na, by ICP-OES (inductively coupled plasma optical emission spectroscopy) in Water Kefir beverages after removing of Water Kefir grains by filtration.

2. Materials and Methods

2.1. Apparatus. pH meter Metrohm mod. 713 was used for pH determination. ICP-OES Optima 7000 DV PerkinElmer was used for quantification of metal ions. Measurements were taken at the following wavelengths: 228.802 nm for Cd, 228.616 nm for Co, 267.716 nm for Cr, 324.754 nm for Cu, 257.610 nm for Mn, 231.604 nm for Ni, 220.353 nm for Pb, 455.403 nm for Ba, 317.933 nm for Ca, 766.490 nm for K, 285.213 nm for Mg, and 589.592 nm for Na.

2.2. Chemical Reagents. Multielement standard solution, 1000 mg/L (CertiPUR®, Merck KGaA), was used, diluted as necessary, to obtain working standards acidified with nitric acid (approx. 0.2% wt/v) for calibration curve; high-quality concentrated nitric acid (70%, ACS reagent, Sigma Aldrich) and ultrapure water obtained using a Milli-Q system (Millipore, Milford, MA) were used.

Solution of metals for Water Kefir colonies was prepared at different final concentrations of Cd (100 μg/L), Co (300 μg/L), Cr (1 mg/L), Cu (5 mg/L), Mn (5 mg/L), Ni (400 μg/L), Pb (200 μg/L), and Ba 1 mg/L (as internal standard) and acidified with nitric acid (10^{-2} M). The pH value of the final solution was 1.88.

Commercial mineral water and commercial sucrose were used for Water Kefir tests. The contents of Ca, K, Mg, and Na in mineral water were determined (Ca = 3.92 mg/L, K = 0.76 mg/L, Mg = 0.76 mg/L, and Na = 2.21 mg/L).

Sodium hydroxide pellets (Reagent grade, Sigma Aldrich), glacial acetic acid (ACS reagent, Sigma Aldrich), and citric acid (ACS reagent, Sigma Aldrich) were used to make buffer solutions as received from commercial suppliers without further purification.

2.3. Labware. The risk of contamination was minimized by using glassware as little as possible and employing new plastic (polypropylene) vessels and pipette tips. All labware was washed with 10% nitric acid solution and rinsed several times with deionized water.

2.4. Water Kefir Samples. Two colonies of Water Kefir with different grains sizes (Figure 1) have been purchased by Kefiring (Kefiring di Fabio Marcolongo, http://www.kefiring.com). The composition of Water Kefir grains of both Colonies 1 and 2 is *Lactobacilli*, yeast, lactic cocci acid bacteria, and *Enterococci*. Both colonies are used to prepare the Water Kefir drink to homemade purpose.

In order to reproduce the domestic preparation conditions, in laboratory, the Water Kefir grains of Colonies 1 and 2 were kept in 1 L commercial mineral water with an addition of 0.5 g of citric acid and 100 g of commercial sucrose (at 20°C). The choice of citric acid is justified by the lemon juice addition according to homemade preparation. The sucrose-citric acid solution has been replaced every 72 hours at 20°C.

2.5. Sample Preparation and Analysis. The water content in the grains was evaluated after 24 hours dehydration in oven at 120°C, resulting to be 85% for sample 1 and 84% for sample 2.

The samples were prepared as follows: (1) 1 g of Kefir grains was added to 10 mL of mineral water and sucrose (5%). (2) 1 g of Kefir grains was added to 10 mL of a metal ion solution (Water Kefir grains/metal solution ratio 1 : 10) prepared with or without sucrose (5%); the initial pH was 1.88, and for varying the pH conditions, a citrate buffer (NaOH 1 M and citric acid $5{\cdot}10^{-3}$ M) was added. 3.5, 4.5, and 6.0 pH values were chosen because of slightly acidic pH of homemade Water Kefir drink (pH approx. 3.5–4.0). The buffer concentration was reasonably diluted ($5{\cdot}10^{-3}$ M) to guarantee a proper initial pH without preventing the metabolic activity of Water Kefir grains (Figure 2). 1 g of Kefir grains was added to 10 mL or 10 g of Kefir grains was added to 10 mL, 1 : 10 or 1 : 1 ratios, respectively, prepared with sucrose (5%) in acetic buffer (NaOH 1 M and acetic acid $5{\cdot}10^{-3}$ M). The initial pH was 4.5.

The pH solution was monitored from 24 hours up to 240 hours (1) or every 24 hours up to 72 hours for the other experimental conditions.

After filtration of Water Kefir grains, ICP analysis of the supernatant solution has enabled to evaluate the amount of biosorbed or bioaccumulated metals. Both heavy metals and mineral water metals (Ca, K, Mg, and Na) were monitored.

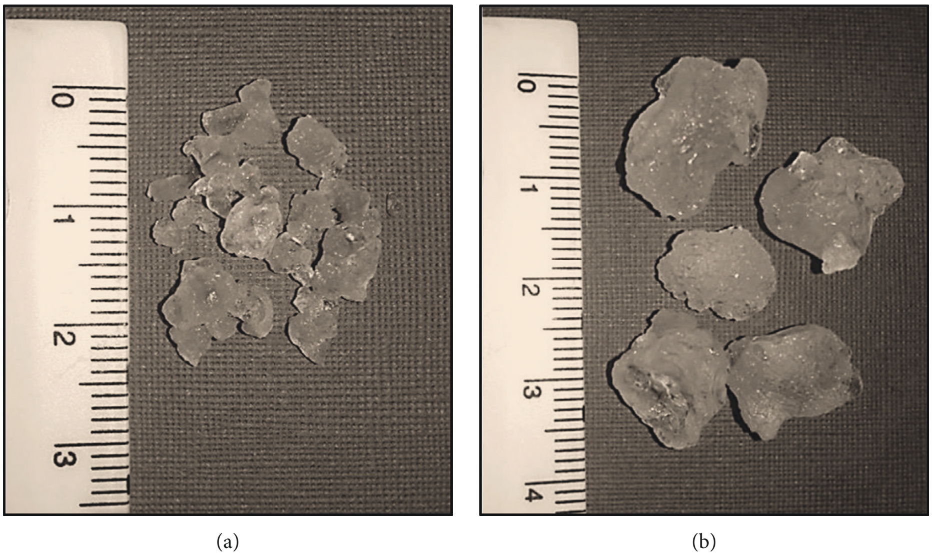

(a) (b)

Figure 1: Water Kefir grains: Colony 1 (a) and Colony 2 (b).

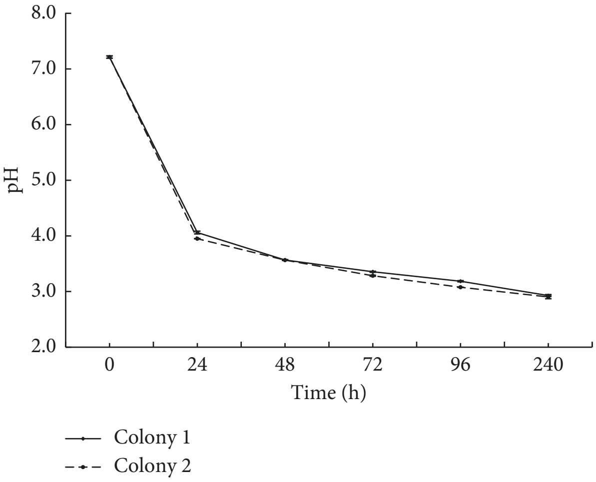


Figure 2: pH trend for Colonies 1 and 2 in mineral commercial water. Conditions: 5% sucrose, initial pH = 7.0, 1 : 10 Water Kefir/metal solution ratio. The results are expressed as the mean of three replications.

The concentration of metal ions was measured every 24 hours up to 72 hours.

An external calibration was performed for quantifying each element. Three replicates were performed at each concentration level, and RSD% values were <2%; therefore, the least-squares regression line was utilized for quantification, and R^2 values of the calibration curves were 0.9900 to 0.9999 depending on the element.

Each experiment had three independent replications of the experiments, and the mean data were used for the evaluation of results. All of them were expressed with a SD. The data dispersion was evaluated by calculating the standard error of the mean (SEM) (for standard errors, μg/L concentrations, see also SI Tables S1 and S2).

A total of 96 samples were studied. All samples were carefully handled to avoid contamination; the appropriate quality assurance procedures and precautions were followed to ensure the reliability of results.

3. Results and Discussion

3.1. pH Trend. The Water Kefir is a weakly acidic beverage, but its pH can greatly change depending on the fermentation time, the addition of other ingredients, and the amount of sugar. The pH trend of a simulated homemade Water Kefir solution, that is in commercial mineral water added with sugar, is shown in Figure 2.

The acid conditions in which Kefir colonies grow were simulated with addition of citric acid. A citrate buffer ($5{\cdot}10^{-3}$ M) was used to change the pH of metal solution from 1.88 to 3.50, 4.50, and 6.00.

Figure 3 shows the trend of pH as a function of time in presence or absence of sucrose for both colonies. The starting pH was 3.50, 4.50, and 6.00, and Water Kefir grains/metal solution ratio was 1 : 10.

Starting from pH = 3.5, 4.5, and 6.0 in presence of sucrose, pH decreased in the first 24 hours due to the

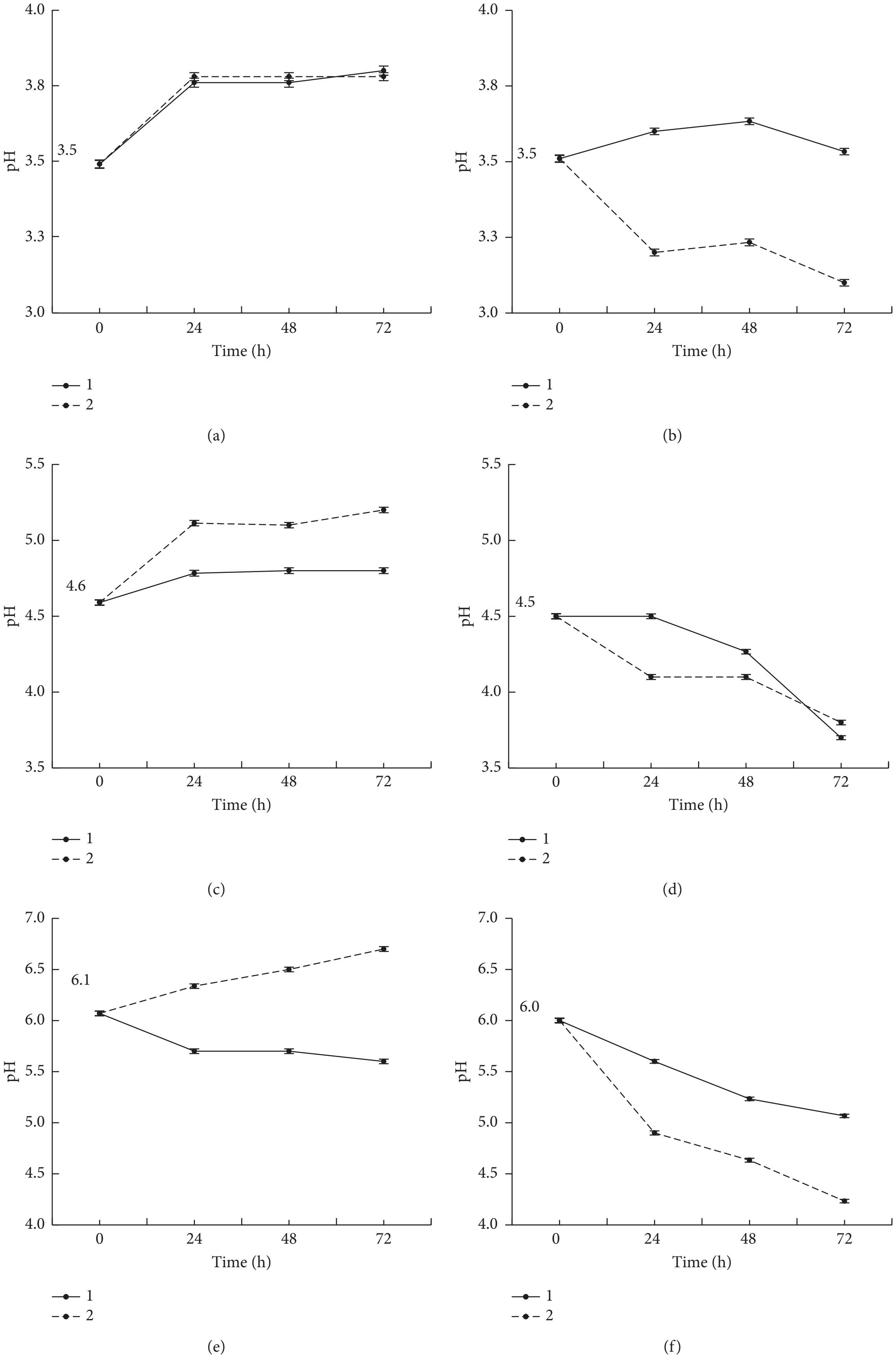


FIGURE 3: pH trend of Colonies 1 and 2 in absence and presence of sucrose (5%). Conditions: $5 \cdot 10^{-3}$ M citrate buffer, initial pH = 3.5, 4.5, and 6.0, and 1 : 10 Water Kefir/metal solution ratio. Left: absence of sucrose. Right: presence of sucrose. $n = 3$.

well-known fermentation process. In the absence of sucrose, pH remained unchanged or slightly increased. A different trend in presence-absence of sucrose is clearly observable, if the other experimental conditions remain constant. The two Water Kefir colonies show a peculiar and different behaviour in the same conditions, probably due to the peculiar size and form of the Water Kefir grains.

3.2. Determination of Metal Ions in the Supernatant Solutions. As reported above, the citrate buffer ($5{\cdot}10^{-3}$ M) was used to change the pH, and the concentration of heavy metals in the supernatant solutions was monitored every 24 hours up to 72 hours for each different initial pH values.

Figure 4 shows the trend of metal concentration (Cr, Ni, Pb, and Cu) as a function of time in presence or absence of sucrose, for both colonies. The starting pH was 3.50, 4.50, and 6.00, and Water Kefir grains/metal solution ratio was 1 : 10.

The concentrations of metal ions did not change significantly in the absence of sucrose (for other metal ions) at the three different initial pH values studied. At initial pH = 6, the concentrations of Ni, Cd, and Cr slightly reduced (lower than 20%), while the contents of Co, Pb, Mn, and Cu were markedly decreased (20–75%). Generally, the concentration trends at 24–48–72 hours were not susceptible to strong modification.

Also in the presence of sucrose, there were not interesting modifications in the concentration of metals after 72 hours (see SI Table S1 for other metal ions). Usually, the content of metals decreased in the first 24 hours and then returned approximately at the initial value in the next 48 hours.

Comparing the two colonies, it is evident that Colony 1 adsorbs/accumulates a greater amount of metal ions than Colony 2 at every initial pH value. This happens probably because Water Kefir Colony 1 acidifies the solution more slowly than Colony 2, so that metal ions are gradually redissolved in the solution. As is common knowledge, metal ion precipitation-complexation equilibria are influenced by pH values [38, 39]. In this work, the pH values are modified by the Kefir metabolic activity.

Moreover, it can be noted that the sucrose presence is essential to have a microbial activity and, consequently, to observe bioaccumulation and/or biosorption phenomena which permit metal ions abatement. However, bioaccumulation/biosorption phenomena due to Water Kefir grains activity could be reduced in the presence of citrate since, as known, citrate anion easily forms metal complexes in solution. Therefore, to evaluate the buffer complexing effect, other samples were prepared with acetate buffer because acetate anion is a weaker ligand compared to citrate in forming metal complexes.

Because of the importance of starting pH value and sucrose presence, in this second data set, acetate buffer ($5{\cdot}10^{-3}$ M) was employed at pH = 4.5 with sucrose. The starting pH value was chosen to make a compromise between the physiological pH of Kefir (3.5–4.0) and the pH value that shows the most successful metal abatement (pH = 6.0). Moreover, in relation to the previously published studies, two Water Kefir grain/metal solution ratios were experimented: 1 : 10 and 1 : 1, that is, 1 g·Kefir/10 ml solution and 10 g·Kefir/10 ml solution. While a 1 : 10 ratio is very similar to the conditions described to prepare home water Kefir beverage (approx. 100 g·Water Kefir grains/1 L·water), the 1 : 1 ratio highlights the metal abatement and is most frequently used in the literature studies.

Figure 5 shows the trend of Cr concentration as a function of time in the presence of sucrose in citrate and acetate buffers, for both colonies. The starting pH was 4.50 and Water Kefir grains/metal solution ratio was 1 : 10. As can be seen, the acetate buffer solution has a more pronounced effect on Cr abatement than citrate one. Similar trends were obtained also for the other metals (see SI Tables S1 and S2 for other metal ions). This result has confirmed what previously reported; that is, citrate anion has more complexing power than acetate anion and so a larger amount of metals is available in solution for absorption/accumulation in acetate buffer.

Two different Kefir/metal solution ratios were studied and compared (1 : 10 and 1 : 1). Figure 6 shows the trend of Cr, Pb, Ni, and Cu concentration as a function of time in the presence of sucrose for both colonies. The starting pH was 4.50 and Water Kefir grains/metal solution ratio was 1 : 10 and 1 : 1 in acetate buffers (see SI Table S2 for all other metal ions). As can be noted, 1 : 1 ratio shows more efficient abatement of metals than 1 : 10 ratio. A possible explanation is that a saturation effect occurs when the absolute quantity of metals increases ten times.

The trends of heavy metal ions abatement as a function of time are reported in Figure 7 for Pb, Cu, Ni, Cr, and Ca (see SI Table S2 for all other metal ions). The starting pH was 4.50 and Water Kefir grains/metal solution ratio was 1 : 10 and 1 : 1 in acetate buffers.

For Cr, Pb, and Mn, the difference between 1 : 1 and 1 : 10 ratio is moderate (10–20%), while for Cu, Ni, Cd, and Co, this difference is much more evident (>20%). Likely, the reason lies in the peculiar Water Kefir colony affinity for each metal.

Figure 8 shows the trend of Ca concentration as a function of time in the presence of sucrose for both colonies. The starting pH was 4.50 and Water Kefir grains/metal solution ratio was 1 : 10 and 1 : 1 in acetate buffers. As can be seen, the Ca concentration increases in the presence of sucrose instead of decreasing as occurs for heavy metals; presumably, this element is replaced by heavy metals during the bioaccumulation/biosorption process. This fact is mainly noticeable for Ca because Na, K, and Mg are at lower concentrations and rarely form coordination complexes.

4. Conclusions

The Water Kefir grains are able to retain heavy metal ions dissolved in aqueous solution. Their metabolic activity is influenced by the surrounding conditions: sugar, contact time, pH, buffer, Kefir grains/metal solution ratio.

The presence of sucrose is necessary to have a microbial activity that induces a metal retention in acid

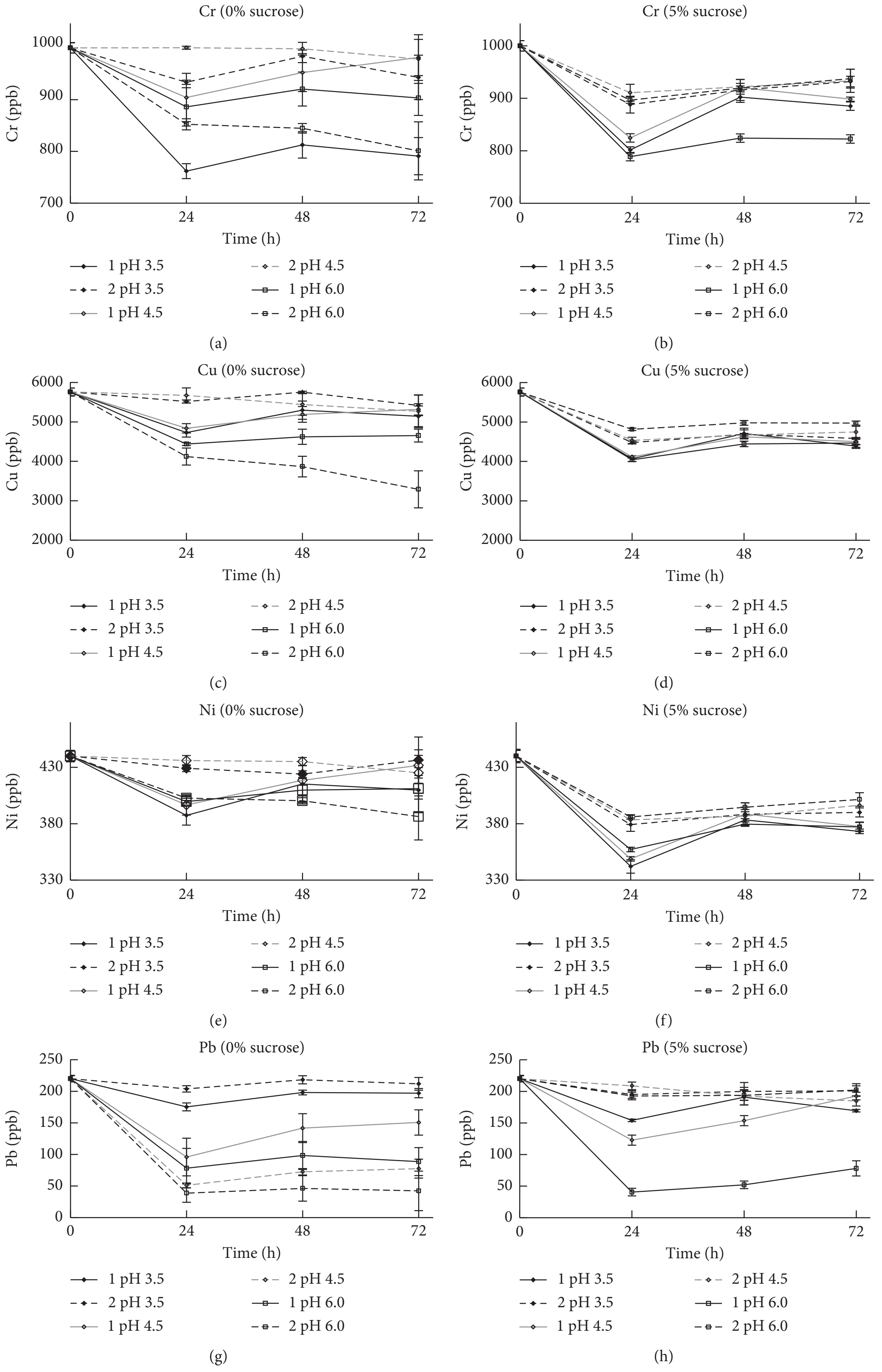


Figure 4: Cr, Cu, Ni, and Pb concentration as a function of time in absence and presence of sucrose (5%) for Colonies 1 and 2. Conditions: $5\cdot10^{-3}$ M citrate buffer, initial pH = 3.5, 4.5, and 6.1, and 1 : 10 Water Kefir/metal solution ratio. Left: absence of sucrose. Right: presence of sucrose. n = 3.

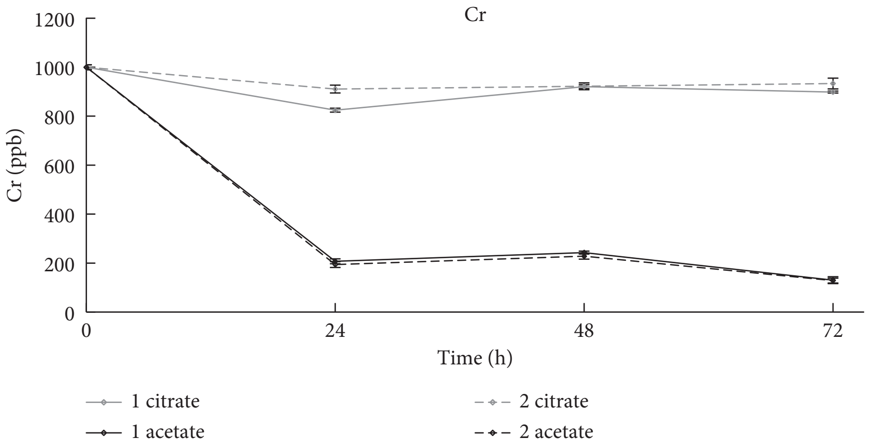
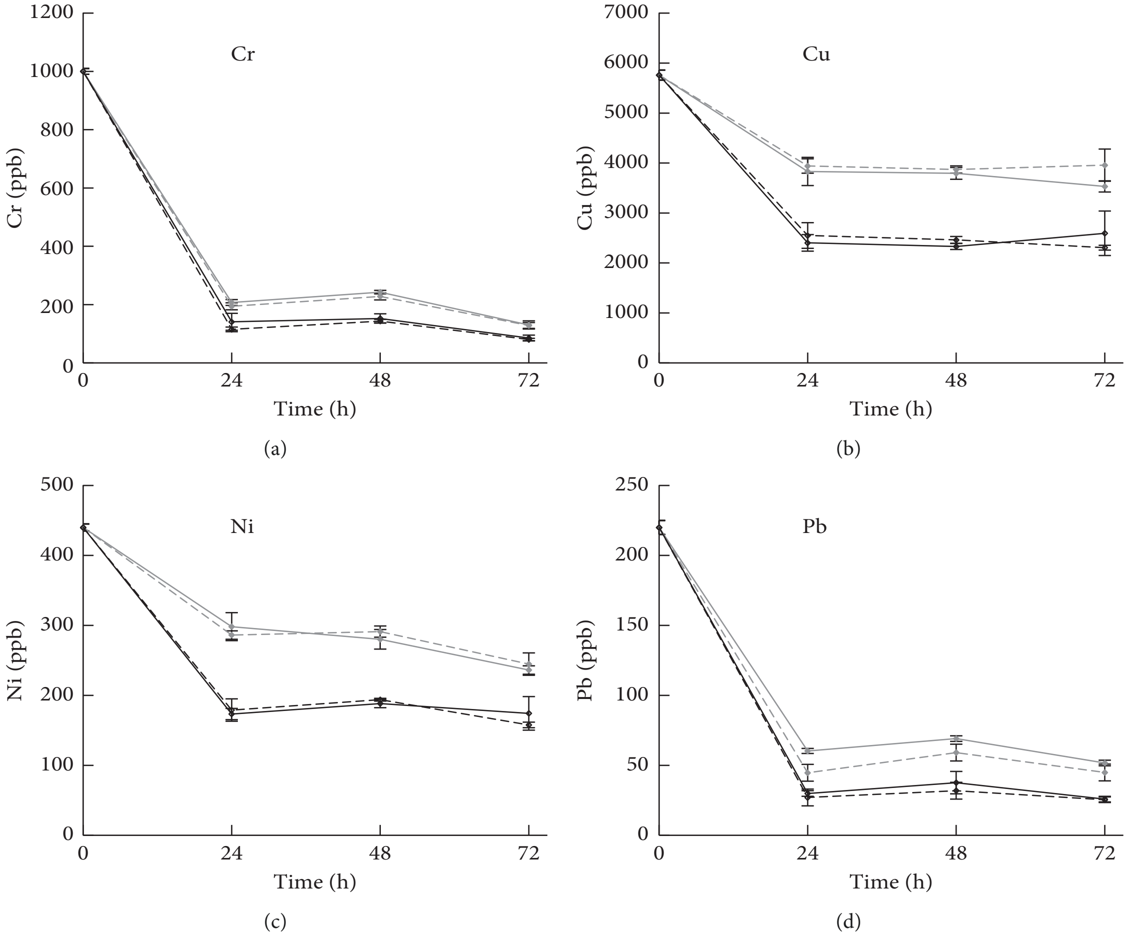


Figure 5: Cr concentration as a function of time in citrate buffer ($5 \cdot 10^{-3}$ M) and acetate buffer ($5 \cdot 10^{-3}$ M) for Colonies 1 and 2. Conditions: 5% sucrose, initial pH = 4.5, and 1 : 10 Water Kefir/metal solution ratio. n = 3.

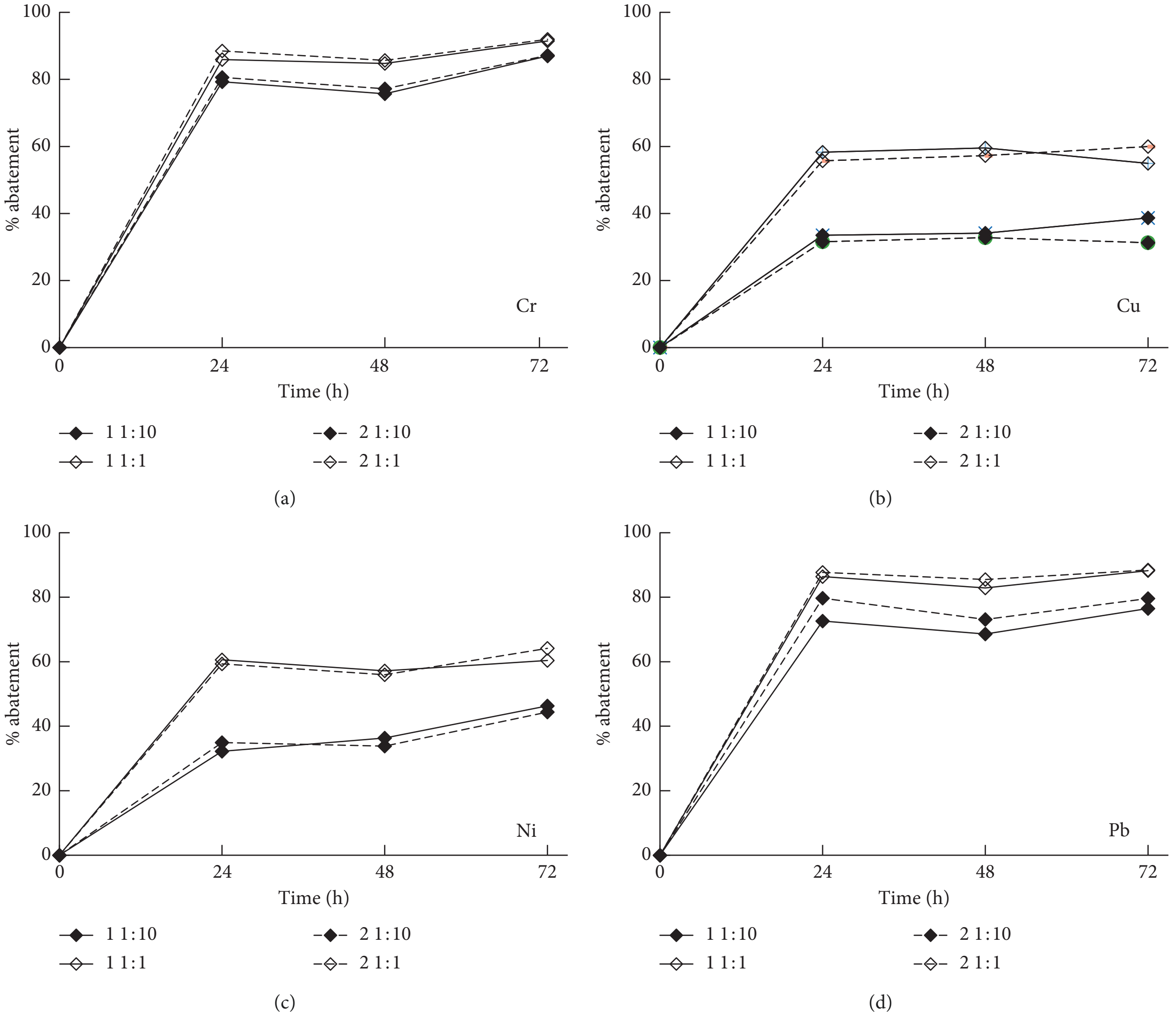


(a) (b) (c) (d)

Figure 6: Cr, Cu, Ni, and Pb concentration as a function of time in acetate buffer ($5 \cdot 10^{-3}$ M) for Colonies 1 and 2 at 1 : 10 and 1 : 1 Water Kefir/metal solution ratios. Conditions: 5% sucrose and initial pH = 4.5. n = 3.

condition. In fact, the reported data show that heavy metal ions are significantly absorbed on the Water Kefir grains surface only in the presence of sucrose, during the metabolic activity.

The most appropriate starting pH is 4.5, which was slightly modified by microorganisms during fermentation resulting in the best performance of abatement of metals after 24 hours. If the initial pH value is too low (3.5), metal ions stay in solution; if the initial pH is too high (6.0), metal ions are quickly adsorbed. When the fermentation activity of the sample decreases, the pH value turns to acid condition and, consequently, metals are redissolved.



(a) (b) (c) (d)

Figure 7: % abatement of Cr, Cu, Ni, and Pb as a function of time in acetate buffer ($5\cdot10^{-3}$ M) for Colonies 1 and 2 at 1 : 10 and 1 : 1 Water Kefir/metal solution ratios. Conditions: 5% sucrose and initial pH = 4.5. *n* = 3.

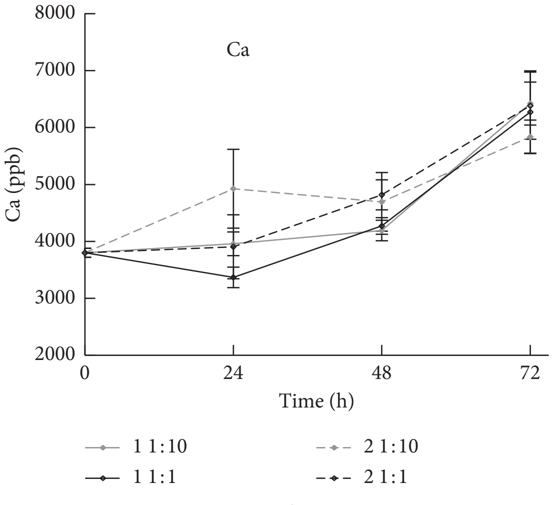


Figure 8: Ca concentration as a function of time in acetate buffer ($5\cdot10^{-3}$ M) for Colonies 1 and 2 at 1 : 10 and 1 : 1 Water Kefir/metal solution ratios. Conditions: 5% sucrose and initial pH = 4.5. *n* = 3.

Precipitation, adsorption, and complexation equilibria are controlled by the buffer type besides the pH value, as mentioned above. Complexing anions like citrate should be avoided because they compete with the biosorption/bioaccumulation phenomena. The acetate buffer has negligible complexing properties.

The 1 : 10 ratio brings lower abatement than 1 : 1 ratio, probably because the metal ion abatement depends also on the absolute quantity of metal ions, and in this condition, a saturation effect occurs on the Water Kefir grains' surface.

Therefore, among the tested experimental conditions, the best combination for pollution abatement is sucrose, 24 hours, pH = 4.5, acetate buffer, Kefir grains/metal solution ratio 1 : 1. In these conditions, the abatement of heavy metals by Water Kefir is particularly effective for Cr and Pb (approx. 70%) and good for Cu, Ni, and Mn (approx. 50%).

In conclusion, Water Kefir grains revealed to be an efficient adsorber/biosorber of metals in the studied and optimized conditions (in particular for Cr, Pb, Cu, Ni, and Mn ions). Moreover, the proposed study represents an efficient procedure to determine the concentration and the abatement of metal ions in fermented drinks whose fermentation is started with solid Water Kefir grains.

The present work demonstrates a possible use of Water Kefir grains on polluted water by heavy metal ions for an efficient and safe purification.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

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Supplementary Materials

Table S1: concentration of other metals in absence and presence of sucrose (5%) for Colonies 1 and 2. Conditions: $5{\cdot}10^{-3}$ M citrate buffer, initial pH = 3.5, 4.5, and 6.1, and 1 : 10 Water Kefir/metal solution ratio. Left: absence of sucrose. Right: presence of sucrose. $n = 3$. Table S2: concentration of other metals in acetate buffer ($5{\cdot}10^{-3}$ M) for Colonies 1 and 2 at 1 : 10 and 1 : 1 Water Kefir/metal solution ratios. Conditions: 5% sucrose and initial pH = 4.5. $n = 3$. (*Supplementary Materials*)

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