# Colour stability of dental restorative materials submitted to conditions of burial and drowning, for forensic purposes

Sergio Augusto de Freitas Vincenti[1], Roberto Cesar Biancalana[2], Ricardo Henrique Alves da Silva[2], Fernanda De Carvalho Panzeri Pires-de-Souza[1].

[1]*Department of Dental Materials and Prosthodontics. Ribeirao Preto School of Dentistry – University of Sao Paulo, Brazil.*
[2]*Department of Stomatology, Public Oral Health, and Forensic Dentistry. Ribeirao Preto School of Dentistry – University of Sao Paulo, Brazil.*

**Corresponding author:**
**ferpanzeri@usp.br**

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KEYWORDS

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## ABSTRACT

The aim of this study was to evaluate the effect of earth and water on the colour stability of tooth-coloured dental restorative materials: composite resin (CR) and glass ionomer cement (GIC). Aiming to distinguish between one and another tooth-coloured material and to estimate the period in which they could be submitted to the factors earth and water, the proposed method may contribute to the proceedings of human identification of victims of burial and submersion in water. Forty bovine incisors were prepared (6 x 6 x 2mm) and restored with CR Filtek™ Z250 XT (3M ESPE™) and GIC Ketac™ Fil Plus (3M ESPE™). After initial colour read-outs (VITA™ Easyshade spectrophotometer), the samples were separated into two groups (n=10), according to the conditions to which they were submitted: simulations of burying and submersion in water, for periods of 1, 3, 6 and 12 months, when new read-outs were taken. The values of colour change ($\Delta E$, $\Delta L^*$, $\Delta a^*$, and $\Delta b^*$) were subjected to 3-way ANOVA statistical analysis, repeated measures, Bonferroni ($p<.05$), and it was verified that both factors produced colour changes in the restorative materials, which were higher for glass ionomer cement ($p<.05$) after 12 months of burial, and 6 months of submersion in water. The authors concluded that the analysis of colour change in the material contributed to the forensic odontology casework depending on the time during which the victim was submitted to the condition of burial or submersion in water.

## INTRODUCTION

Floods are often cited as being the most lethal of all natural disasters; during the twentieth century, floods killed at least eight million people.[1] On December 26, 2004, a major earthquake and the resulting tsunami killed more than 230,000 people in 12 countries on the shores of the Indian Ocean.[2-4] The devastating consequences of that event have led to the most significant international effort undertaken so far to identify victims of natural disasters.[4] On January 2011, a devastating tropical storm hit the mountain area of Rio de Janeiro State in Brazil, resulting in flooding and mud slides and there were 845 immediate deaths. One year after the disaster, the number of dead and missing persons was estimated to be 1,300.[5]

In such cases, the search and rescue of victims may require extended time due to the local climate and geographical conditions.[6-8]

Also, bodies may be entirely or partially decomposed or fragmented, making the proceedings of human identification a challenge for the forensic expert teams.[9-12] Given the poorly preserved remaining tissues or lack thereof, the feasibility of fingerprint analysis can be forgone[7, 8, 13, 14].

The durability of teeth is a feature that makes forensic dentists regular participants in forensic investigation[3, 15]. The teeth can stand up to most post mortem events that can disrupt or alter other body tissues.[10, 11, 13] Teeth are often the only findings that can be analyzed and, in these cases, Forensic Odontology becomes the most viable, practical, fast, if not the only possible identification method available.[9, 12] If there are any dental restorations, the restorative dental materials are exposed to the same agents as the teeth, for example, earth and water. Thus, these agents cannot destroy the teeth but could modify the dental restorative materials.

Restorative dental materials are subject to intrinsic changes over time; moreover, their physical and mechanical properties characterize and distinguish them from the others.[16] Studies have been carried out with the aim of identifying changes occurring in dental materials when subjected to extraordinarily high[7, 17] or low[8] temperatures; but to date, there have been no studies on the changes caused by earth and water in these materials. Among the possible changes in the physical and mechanical properties of restorative dental materials, the colour stability has been studied, to establish which agents are responsible for the changes, as well as to estimate the period during which the materials were exposed to such conditions.[7, 8]

In this context, the authors considered it essential to carry out an experimental study to learn more and improve the knowledge base about the changes that restorative materials undergo when exposed to the effect of the earth and water. Thus, this study may contribute to the human identification proceedings of forensic odontology teams involved in Disaster Victim Identification (DVI) cases. Thus, the aim of this study was, by the simulations of burial and submersion in water, to evaluate the effect of earth and water on the colour stability of the dental restorative materials most commonly used in the daily dental practice.

## MATERIALS AND METHODS:

Forty sound bovine incisors were restored in the central region of the buccal surface and randomly separated into two groups according to the restorative material used (Table 1). After restoring the teeth, the first colour read-outs were taken, using a portable spectrophotometer (Easyshade™, VITA, Bad Säckingen, Germany). Three colour read-outs were taken, according to the CIE $L^*a^*b^*$[18] scale, and the mean of these values was considered the initial value. The CIE $L^*a^*b^*$ scale (Commission Internationale de L'Eclairage) consists of three Cartesian coordinates in which $L^*$ indicates lightness, $a^*$ indicates the green-to-red shade, and $b^*$ indicates the blue-to-yellow shade.[18]

The teeth restored with each material were randomly separated into groups (n=10), according to the environment to which they were submitted: burial or submersion in water. To simulate the burying environment, the teeth were inserted into an excavated 4 feet deep hole, and covered by earth. For the simulation of submersion in water, the teeth were inserted into a nylon bag with open and reticular wefts and chained from a fixed point on the shore of a lake and then submerged into that lake within a bird cage.

After periods of 1, 3, 6 and 12 months, the samples were withdrawn from the environmental conditions and new colour readings were performed by the same operator who carried out the initial readings. The colour stability of the restorative materials was calculated by the formula:[18]

$$\Delta E = \sqrt{(\Delta L^*)^2 + \Delta a^*)^2 + (\Delta b^*)^2}$$

Where $\Delta L^* = L^*_f - L^*_i$; $\Delta a^* = a^*_f - a^*_i$; $\Delta b^* = b^*_f - b^*_i$, being $L^*_i$, $a^*_i$ e $b^*_i$ referred to as the initial read-outs, and $L^*_f$, $a^*_f$ e $b^*_f$ as final read-outs for colour coordinates. The colour values (ΔE) and the changes in the coordinates ($\Delta L^*$, $\Delta a^*$ and $\Delta b^*$) were analyzed according to 3-way ANOVA, repeated measures, Bonferroni, p<.05, to compare the materials, the environments and the periods tested.

**Table 1**: Materials used, commercial brands, manufacturers, colour and method of restoration.

| Category | Brand Name | Manufacturer | Colour | Method of restoration (Clinical steps) |
|---|---|---|---|---|
| **Composite resin** | Filtek™ Z250 XT | 3M ESPE™, Sumare, SP, Brazil | A3 | 1. Acid etch (37% phosphoric acid, Alpha Etch DFL™, Rio de Janeiro, RJ, Brazil) for 15 seconds, washing, and drying;<br>2. Bonding system application (Adper Single Bond 2, 3M ESPE™, Sumare, SP, Brazil) and light curing (Ultralux EL, Dabi Atlante™, Ribeirao Preto, SP, Brazil) for 10 seconds;<br>3. Material insertion in increments and light curing for 20 seconds;<br>4. Finishing and polishing (flexible discs Sof-Lex™ Pop-On, 3M ESPE™). |
| **Glass ionomer cement** | Ketac™ Fil Plus | 3M ESPE™, Sumare, SP, Brazil | A3 | 1. Powder/liquid (1:1) agglutination up to 1 minute;<br>2. Material application in increments until the cavity filling. |

## RESULTS

The comparisons of the ΔE mean values are shown in Table 2. There was no statistically significant difference (p>.05) in colour change for composite resin (CR), irrespective of the period that the samples were submitted to the agents. The most significant (p<.05) change for the glass ionomer cement (GIC) occurred after 12 months, in both environments. Water produced a higher change (p<.05) than earth, after 6 months for CR and GIC, and 12 months for GIC (Fig. 1).

The comparisons of the ΔL* mean values are shown in Table 3. Both materials lost luminosity when submitted to submersion in water for 1 month, a different result (p<.05) in comparison with the same period of burial. For GIC, the most significant change (p<.05) was verified after 12 months of submersion in water. The most significant difference (p<.05) between CR and GIC occurred after 1 month of burial, and 3 months of submersion in water (Fig. 2).

The comparisons of the Δa* mean values are shown in Table 4. There was significant difference (p<.05) for the CR submerged in water for 12 months, and for CR after 6 months, and GIC after 12 months when submitted to both environments. The most significant difference (p<.05) between CR and GIC occurred after 1 month of burial, and 1 and 12 months of submersion in water (Fig. 3).

The comparisons of the Δb* mean values are shown in Table 5. There was a higher change in both materials after 6 months of burial and submersion in water, with statistically different results (p<.05) for CR. The most significant difference (p<.05) between CR and GIC occurred after 1 month of burial, and 1 and 6 months of submersion in water (Fig. 4).

Representative photographs of the colour changes, before and after the submission to the proposed tests, are shown in Figs. 5 and 6. It was verified a change in the colour of CR after 1 month of burial, remaining stable after 3 months. After 6 and 12 months, new colour changes were observed. Regarding GIC, despite the apparent loss of brightness after the first month, the most significant colour changes were seen after 3 months and were maintained until 12 months of burial. A significant change in the CR colour was observed after 1 month of submersion in water, remaining stable after 3 months. The highest change was verified after 6 months, looking much clearer in relation to the other periods. Concerning GIC, there was a permanent darkening after 1 and 3 months, but the most significant change in the colour was observed after 6 months, the restoration was very whitish.

**Table 2**: Comparison of means of ΔE (standard deviation) of the agents, in different periods, for the same material.

| Material | Periods (months) | Earth | Water |
|---|---|---|---|
| CR | 1 | 3.08 ±0.69 aA | 3.64 ±1.55 aA |
| | 3 | 1.59 ±0.52 aA | 2.07 ±0.73 aA |
| | 6 | 4.01 ±1.13 bA | 5.28 ±1.52 aA |
| | 12 | 2.31 ±0.84 aA | 2.17 ±0.93 aA |
| GIC | 1 | 2.94 ±1.41 aB | 7.23 ± 3.82 aC |
| | 3 | 4.60 ±3.99 aB | 4.96 ± 2.50 aC |
| | 6 | 5.24 ±3.68 bB | 12.30 ±7.50 aB |
| | 12 | 12.17 ±6.61 bA | 24.07 ±5.42 aA |

Different letters, lower case letters on the line and capital letters in the column indicate statistically significant results (p<.05).

**Figure 1**: Graphic representation of the comparison of ΔE between CR and GIC. Horizontal lines above the bars indicate statistically significant results (p<0.05).

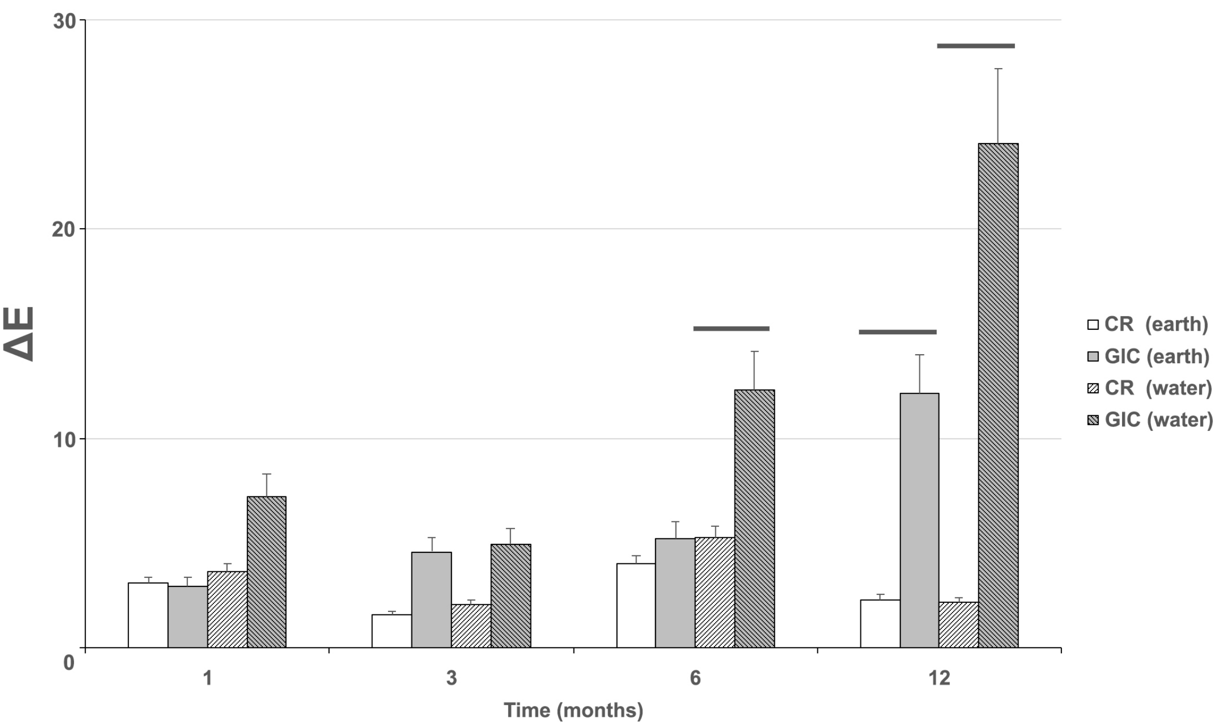

**Table 3**: Comparison of means of ΔL* (standard deviation) of the agents, in different periods, for the same material.

| Material | Periods (months) | Earth | Water |
|---|---|---|---|
| CR | 1 | 1.19 ±0.93 bA | -1.88 ±1.53 aA |
| | 3 | 0.27 ±0.70 aA | 0.48 ±1.17 aA |
| | 6 | 1.58 ±1.02 aA | 1.53 ±0.95 aA |
| | 12 | 0.35 ±0.82 aA | -0.10 ±0.89 aA |
| GIC | 1 | 0.00 ± 2.71bc | -6.87 ± 4.00 aC |
| | 3 | -3.76 ±4.41 aB | -3.86 ± 3.24 aC |
| | 6 | -2.96 ± 4.42 bBC | -11.37 ±7.88 aB |
| | 12 | -11.62 ±6.54 bA | -23.44 ±5.84 aA |

Different letters, lower case letters on the line and capital letters in the column indicate statistically significant results (p<.05).

**Figure 2**: Graphic representation of the comparison of ΔL* between CR and GIC. Horizontal lines above the bars indicate statistically significant results (p<0.05).

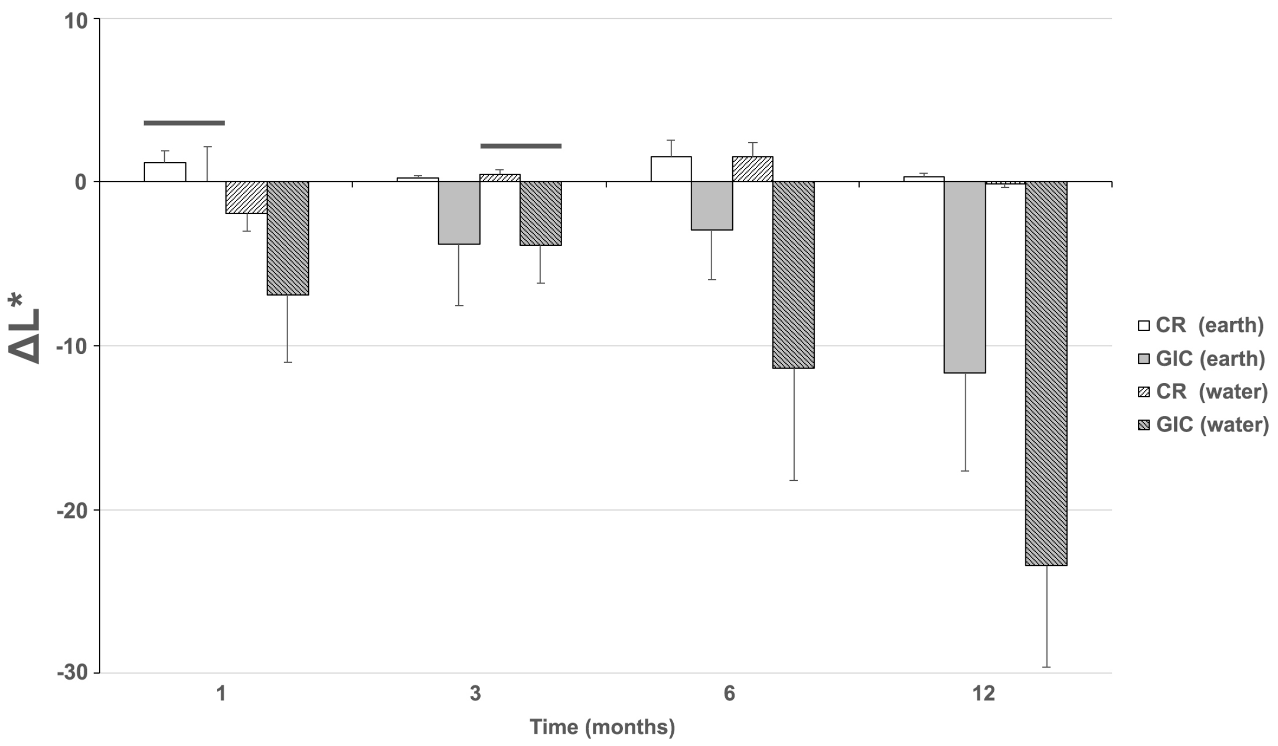

**Table 4**: Comparison of means of Δa* (standard deviation) of the agents, in different periods, for the same material.

| Material | Periods (months) | Earth | Water |
|---|---|---|---|
| CR | 1 | 1.98 ±0.26 aA | 1.51 ±0.47 aB |
| | 3 | 1.18 ±0.41 aB | 1.23 ±0.33 aB |
| | 6 | 2.34 ±0.48 aA | 2.36 ±0.57 aA |
| | 12 | 1.76 ±0.51 aAB | 1.17 ±0.58 bB |
| GIC | 1 | 0.01 ± 0.68 aC | 0.40 ± 0.61 aC |
| | 3 | 0.31 ± 0.87 aC | 0.67 ± 0.84 aC |
| | 6 | 1.34 ±1.77 aB | 1.77 ±0.99 aB |
| | 12 | 2.60 ±1.74 aA | 3.27 ±0.96 aA |

Different letters, lower case letters on the line and capital letters in the column indicate statistically significant difference (p<.05).

**Figure 3**: Graphic representation of the comparison of Δa* between CR and GIC. Horizontal lines above the bars indicate statistically significant results (p<0.05).

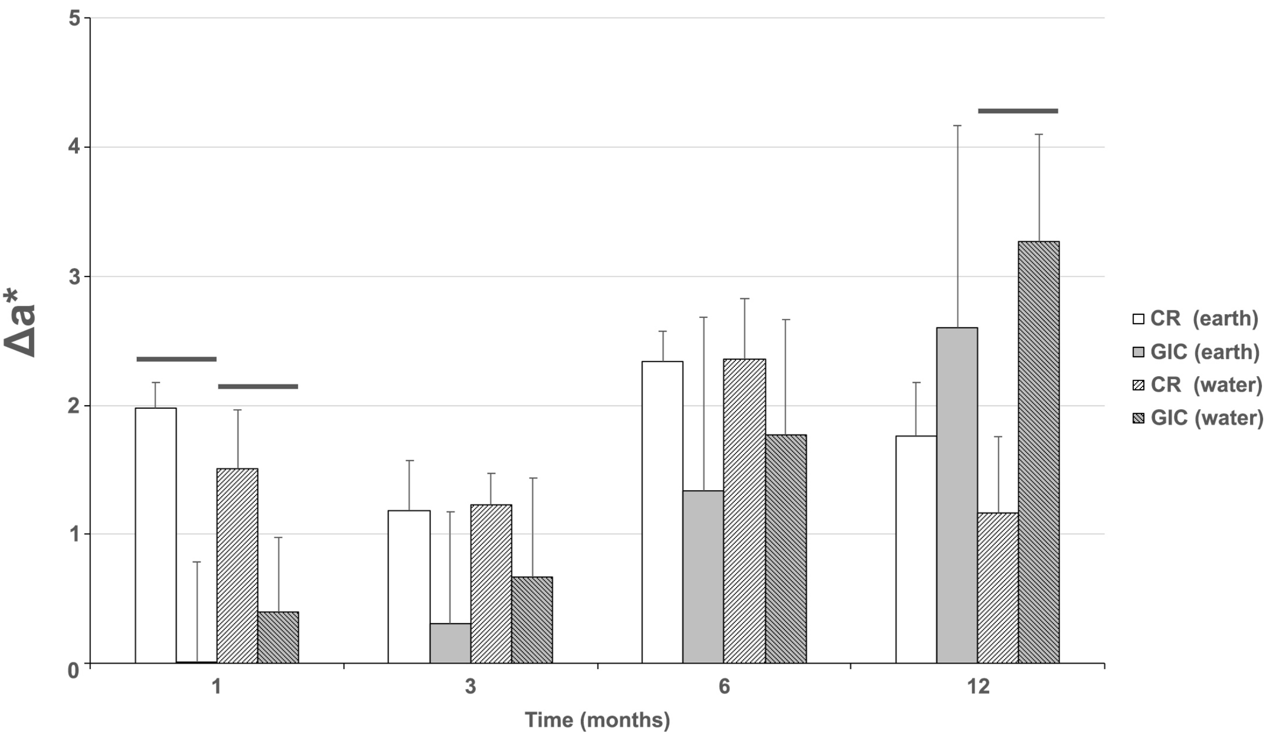

**Table 5**: Comparison of means of Δb* (standard deviation) of the agents, in different periods, for the same material.

| Material | Periods (months) | Earth | Water |
|---|---|---|---|
| CR | 1 | 1.85 ±0.62 aAB | 2.38 ±1.35 aB |
| | 3 | 0.36 ± 0.81 aC | 1.15 ±0.64 aB |
| | 6 | 2.70 ±0.96 bA | 4.42 ±1.28 aA |
| | 12 | 1.10 ±0.87 aBC | 1.31 ±1.23 aB |
| GIC | 1 | -1.16 ± 1.50 aC | -1.64 ±0.85 aB |
| | 3 | -0.97 ± 1.53 aC | 0.89 ±2.12 aA |
| | 6 | 1.43 ±2.72 aA | 1.66 ±3.34 aA |
| | 12 | 0.34 ±2.17 aB | 0.17 ±3.99 aAB |

**Figure 4**: Graphic representation of the comparison of Δb* between CR and GIC. Horizontal lines above the bars indicate statistically significant results ($p<0.05$).

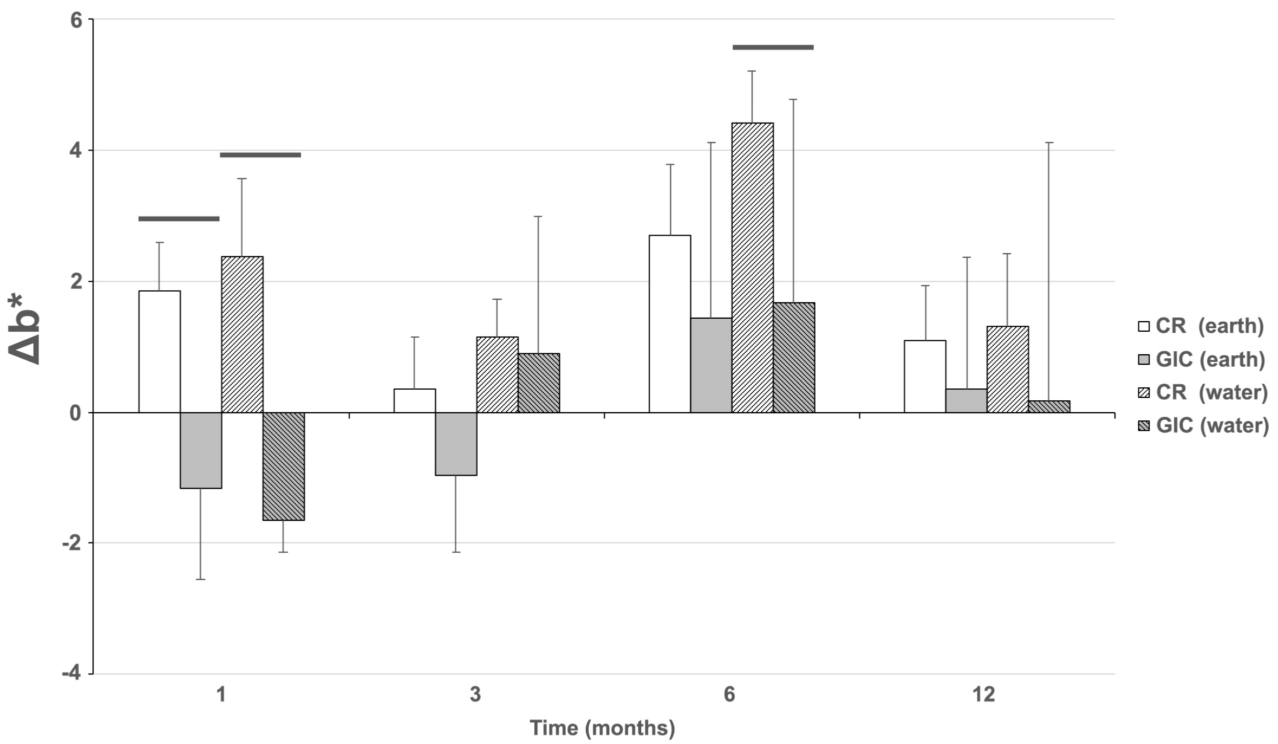

**Figure 5**: A photographic comparison of the restorations of CR (above) and GIC (below) submitted to different periods of burial.


**Figure 6**: A photographic comparison of the restorations of CR (above) and GIC (below) submitted to different periods of submersion in water.

## DISCUSSION

In cases of a delayed discovery of a body, it is not possible to estimate the time of death by analysis such as cooling the body, cadaveric rigidity or hypostasis, for example.[19] However, the study of the physical and mechanical properties of the materials is relevant to distinguish between them and hence to assist in the identification procedure of victims.[7, 8]

The analysis of the colour change of the dental restorative materials, at different periods, may contribute to the forensic expert works, when the victims are found buried or submerged in water, providing information that allows distinguishing the materials found in the oral cavity. Such discrimination may help in the comparison between the ante-mortem information (in the dental records of the subject, if any) with the post-mortem information, collected and reported by the forensic odontologists.

Colour matching is a routine procedure in daily dental practice, and it is critical to the success of the restoration.[20] Therefore, this information must be recorded on the patient's chart. Moreover, dentists have a medico-legal obligation and a social responsibility to exercise great care in the documentation of the treatment procedures[21]. The quality of the ante-mortem dental records, which serve as evidence, is very important in forensic work and deficient ante-mortem charting could hamper forensic odontology casework.[2, 12, 21] Records with accurate and complete data are obviously much more likely to be matched.[3]

Thus, this study aimed to evaluate the effect of earth and water on the colour stability of the dental restorative materials most commonly used in the daily dental practice.[22] The authors started with the null hypothesis that the agents would not produce changes in the colour stability of each material, irrespective the period; and that the differences between the materials could distinguish between them. The results indicated that the null hypothesis could not be accepted because there were significant ($p<.05$) changes in the colour of the materials. However, the second hypothesis can be accepted because that difference allows distinguishing between the materials, according to the method proposed.

The colour changes were analyzed using a portable spectrophotometer, that is the most accurate and reliable method for this type of analysis because it does not allow for subjective evaluations and avoids errors in the colour interpretation. The instruments are more accurate than the eyes and the inconsistent perception of colour by the observers.[23]

Bovine teeth were used as the substrate for the restorations, because of the bioethical issues.[24, 25] They show similarities with the human teeth, as the direction of the enamel prisms, an equivalent percentage by weight of calcium, and protein matrix formed by the same amino acids. [24] The literature shows that bovine dentine is feasible for adhesion, providing adequate bond strength when compared to human dentine.[25] Moreover, this study evaluated changes occurred in the restorative materials, not dental structures.

The periods established for the analysis of this study (1, 3, 6, and 12 months) were determined after other studies showed that the majority of missing corpses were found up to 1 year after their disappearance.[26, 27] Furthermore, the Council of Europe[28] recommends that when the circumstances of a person's disappearance are reasonable to conclude that death is likely, searches must be closed within a maximum of one year after the disappearance, as it was done in the tragedy that occurred in Rio de Janeiro, Brazil, in 2011.[5]

The results showed that composite resin (CR) had significantly different colour changes ($p<.05$) than GIC after 1 year of burial and 6 months of submersion in water. Characteristics of the chemical structure of the composites, as well as the composition of the resin matrix, may interfere with their colour stability.[16] The resin matrix is susceptible to water penetration, which is associated with the discolouration of the composite, so that the greater the percentage of resin matrix in its composition, the greater the possibility of colour change.[29]

The composition of the resin matrix can also have an effect on water absorption because the monomer TEGDMA absorbs more water than UDMA, which in turn absorbs less water than Bis-GMA.[29] The higher predisposition to water absorption of the monomer TEGDMA increases the solubility of the formed polymer, giving these composites less colour stability when combined the monomers Bis-GMA and TEGDMA, due to the increase in the free volume of the formed polymer and, hence, the larger room for the water molecules to diffuse into the polymeric structure. [29] But the hydrolytic degradation reaches its limit with the saturation of the polymer network, and the structure of the composite achieves stability. Thus, the changes in mechanical properties cease, and no longer change over time.[16, 29]

The glass ionomer cement (GIC) is synthesized by an acid-base setting reaction between the polyacrylic acid and the calcium aluminum fluorosilicate glass particles. The resulting product is glass polyalkenoate. It presents a slow setting reaction and initial sensitivity to water loss (syneresis) and gain (soaking).[30] Thus, because it is susceptible to imbibition caused by submersion in water, GIC may present a more significant colour change, as the results showed. There was also significant colour change when the material was buried, especially when compared to CR.

The comparison of the coordinates ($L^*$, $a^*$, and $b^*$) allows analyzing the contribution of each of them to the total colour change of the samples ($\Delta E$). The $L^*$ value, lightness indicator, increased after burying the CR, showing that the restorations became clearer at any period. For the GIC, this value decreased, suggesting a loss of luminosity and consequent darkening of the restorations. When subjected to the action of water, both materials lost luminosity, mainly the GIC, because of its susceptibility to imbibition.[30]

The $a^*$ coordinate values increased, suggesting saturation of the red hue, verified by the changes in CR, up to 6 months, and in GIC, after 12 months. Possibly, this result was caused by the earth colour of the burial place. In turn, the change in the $b^*$ coordinate was positive for both materials, what explains the yellowing of the restorations.

Distinguishing between the tooth-coloured restorative materials would be one of the possibilities in which this study could contribute to forensic works of human identification. Thus, the results allow us to state that the statistically significant colour change ($p<.05$) is a determining factor for this discrimination.

Another significant contribution would be to provide information about the periods in which the materials were submitted to the action of earth and water, and thus help with the estimation of the time lapse between death and the necroscopic examination of the victim. Thus, this study could correlate the existence of dental remnants with a chronology of the thanatological changes, becoming a practical and accessible tool to help in establishing the medical and legal causa mortis. It could also provide clues as to whether the corpse suffered ante-mortem or post-mortem wounds or displacements, thus contributing to the investigations and even positively identifying or excluding suspects. The results show that the colour change ($\Delta E$) of the materials is time-dependent, that is, the longer the agent acts, the greater the discrimination potential of the materials, from 6 months. This is justified by the inherent properties of each material, described above.

## CONCLUSION

The authors concluded that burial and submersion in water produce changes in the colour of the tooth-coloured restorative dental materials, contributing to Forensic Odontology in procedures of human identification, depending on the period that the victim was submitted to these conditions.

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