

CONSERVATION-RESTORATION OF A BOTANICAL MUSEUM FLUID COLLECTION: PRACTICE AND RESEARCH

M. DANGEON,^{1,2} E. CORNET,² AND L. BRAMBILLA²

¹*Botanisches Museum of University of Zürich, Switzerland
mariondangeon@hotmail.com*

²*Haute Ecole Arc Conservation-Restoration, HES-SO University of Applied Sciences and Arts
Western Switzerland*

Abstract.—The collection of the Botanical Museum of the University of Zürich is an academic collection assembled from 1891 to the end of the 20th century (1992 for the last inventoried item). Preserved plants come from all over the world (40 countries) and include all categories of existing Plantae (algae, lichens, fungi, higher plants, bacteria). The fluid collection, largely neglected since 1976, shows significant degradation. The main problem is loss of preservative fluid due to leakage of the jars and aging of the seals. Another issue is the discoloration of the specimen fluids. These issues led to a research project titled FLUIDIS, which aimed to explore different preservative solutions and their impact on the discoloration of plant specimens. Conservation-restoration work was carried out on the jars of the “Professor Ernst Collection.” Topping up of was necessary for the entire collection. Restoration was performed after opening the containers and identifying the fluid. The specimens were consolidated, repaired, and mounted when necessary, then gradually put back into alcoholic solutions and finally sealed. An overall intervention protocol was established for the treatment of the entire botanical fluid collection. Its application, however, requires a careful study of each specimen.

Key words.—botanical collection, conservation, discoloration, ethanol, fluid-preserved, formaldehyde, glycerol, plant specimen, preservative, restoration.

INTRODUCTION

The preservation of natural history specimens in fluid dates to the 17th century (Simmons 2014). The first recipes are mostly unknown, referred to as “liquor” in the literature. Over the years, the use of an alcohol-based preservative fluid with various quantities of other additives became a common practice. These preservation techniques were derived from oral tradition or old recipes, without systematic scientific study on how the choice of constituents and their relative quantity affected the preservation of the specimen (Simmons 2014).

This article presents the results of conservation-restoration work carried out on the University of Zürich’s fluid-preserved botanical collection. The article is composed of three parts: the first section presents the results of a report conducted on the collection in 2016 to determine its state of conservation, the second section describes conservation-restoration interventions performed on the specimens of the collection, and the third section describes an experimental approach to examine the problem of plant discoloration.

The Botanical Museum of the University of Zürich was created at the instigation of Hans Schinz in 1895. Initially, there were two institutes with collections, and these were combined in 1976. The collections of the botanical museum include multiple objects, such as 3D pedagogical models, seed collections, pictures, posters, microscopic slides, and fluid-preserved plants. The collections were gradually forgotten after 1976 and were rediscovered in the 2000s. Since its inception, the entire collection has been moved six times.

The fluid collection, numbering 617 specimens, is now housed in Villa Rainhof, a building dating from 1867 that is currently inside the Botanical Garden established in 2008. The two collection storerooms are located in the basement of the Villa. This storage space was not designed to accommodate collections and has deficiencies from a conservation point of

view. The lack of climate control and the presence of high relative humidity (between 80% and 100% in spring and summer) led to two successive mold infestations between 2008 and 2010. These two infestations caused severe damage to the collection of fluid-preserved plants in particular through loss of information on labels and fluid evaporation due to the degradation of jar seals. As a result of this damage, it was decided to discard about a third of the collection that was considered irreparably damaged or for which no qualified personnel could ensure preservation (Dangeon 2016).

For the remaining jars of the fluid collection, the aging of the seals caused fluid evaporation that endangered the physical integrity of the objects. Generally, in fluid collections, jars are the most frequent object requiring intervention, requiring replacement of seals and the replacement or topping off of the fluid (Cuisin 2016). These interventions may not only cause further damage to the contents but also change the nature of the fluid collections. In fact, if jars, labels, and/or solutions are replaced with more modern materials, part of the original historical information is lost, and the cultural value of the specimens or artifacts can change. In some cases, this replacement is necessary for health and safety reasons, as is the case for replacing formaldehyde with less harmful solutions. Documented protocols are needed for tracking the conservation of such specimens, but this is not often the case in historical collections.

In 2015, at the instigation of the new curator, Dr. Christiane Jacquat, the Botanical Museum's fluid collection was recognized for its scientific and historical value. Decisions for its preservation were made, and conservation-restoration work was initiated, starting with the so-called Ernst Collection of the collector Alfred Ernst, who, through his various expeditions, notably to Java, contributed enormously to the collections of the Botanical Museum.

CONDITION REPORT OF THE UNIVERSITY OF ZÜRICH'S BOTANICAL WET COLLECTION

Since this collection of plants in fluid was abandoned for an extended period, a detailed evaluation of its state of conservation was carried out in 2016. This condition report was based on the evaluation of the appearance of the specimens, the fluid level, and the condition of the jars, sealing materials, and labels. All of these parameters were categorized according to the flowchart proposed by Moore (1999).

It is well known that jar sealing is the weak point of the fluid conservation system (Simmons 2014). Proper sealing maintains the level and concentration of the preservation liquid. Improper seal mounting, poor choice of seal material, seal aging, or interaction with the external (temperature, relative humidity, light) and internal (solvent vapors) environments are the main causes for lack of performance that result in evaporation and/or fluid leakage (Moore 2007, Simmons 2014, Cuisin 2016). When a seal fails, the degradation of material results in loss of the hermetic seal (Dangeon 2016). As a result, exposure to oxygen and to changes in relative humidity made possible by continued fluid evaporation is likely to cause irreversible damage to the specimen. Consequently, the identification of the different seals was the first step of the condition report. A wide range of sealing materials were documented in the collection. The most common were wax, pine resin, masking tape (paper-based tape), rubber gaskets in combination with a screw or flip-top system, and silicone. A very common material was the rubber seal gasket typically found on glass jars used for preserving food. In this collection, degradation of the rubber gaskets generally presented as two phases: either powdery and dry or shiny and almost oozy. In both cases, we assumed that the rubber had degraded and no longer formed an adequate seal. Wax used in the sealing of the jars exhibited a granular appearance and demonstrated little adhesion. The framing tape in the collection was composed of pine resin that showed loss

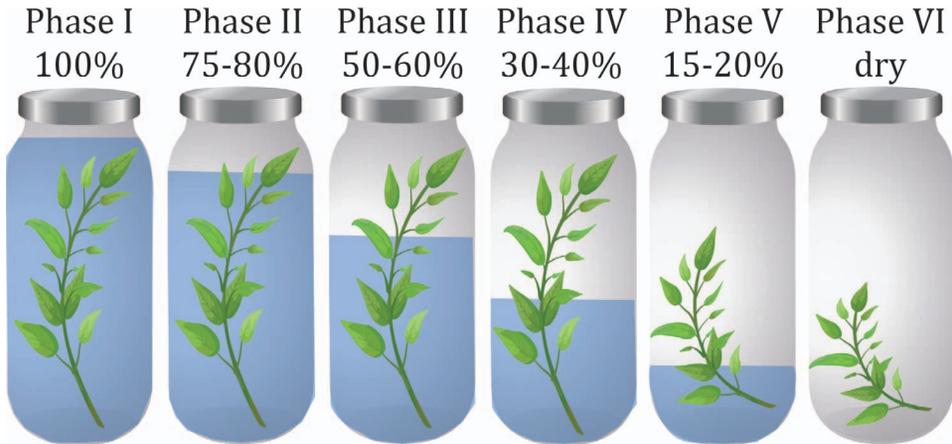


Figure 1. Different states of specimen emersion due to liquid evaporation (©Adapted from Cuisin, 2016).

of adhesion and no longer formed an airtight seal. The pine resin had oxidized, yellowed, and become very brittle.

Because the collection is also used for research purposes, some jars had been opened and resealed. Some of these jars appeared to be resealed with temporary materials that are not suitable for sealing collections in fluid over the long term. These materials are perishable over time, and the temporary seals were never replaced with permanent treatments. Overall, the aging of the sealing material in the collection led to changes in the properties of the seal as described by other researchers, including loss of cohesion, loss of adhesion, porosity, and loss of flexibility (Moore 2010). The different seals visually identified in the Zürich collection were no longer hermetic and allowed for the evaporation of the preservative solution.

The second concern was to evaluate the nature and condition of the fluid preservative itself. The main risk with liquids for preservation is the evaporation linked to sealant failure (DeWolf 1968). To evaluate the extent of evaporation in the entire collection, a visual sight check of the 617 jars was conducted. This sight check was based on a technique developed by Jacques Cuisin (French Natural History Museum). This technique assigns six different evaporation phases, ranging from stage 1 as a full jar of fluid to stage 6 as a totally dry jar (Fig. 1). The aim is to determine the quantity of the remaining fluid by instant visual inspection. This assessment identifies those jars that need to be prioritized and filled as quickly as possible and those that could not be saved without time- and resource-consuming methods. In this collection, we observed all the different stages of evaporation from stage 1 to stage 6. Intervention is necessary from stage 3 to stage 5, and we found that half of the collection required intervention and was at risk. (Fig. 2b, c). Half of the collection required the addition of preservative to reestablish the appropriate fluid level.

Another issue was the change of the optical properties (such as color, transparency, separation of phases, and turbidity) of the preservative fluid, resulting in different stages from clear to complete opacity. The discoloration of specimens, leading to increasing opacity of the preservation fluid, is a phenomenon encountered in all fluid-preserved collections, but it is particularly prevalent in botanical collections due to a higher affinity between preservative fluids and the organic pigments present in plants. For many jars, the condition of the specimen could not be ascertained without opening the jar because the opacity of the

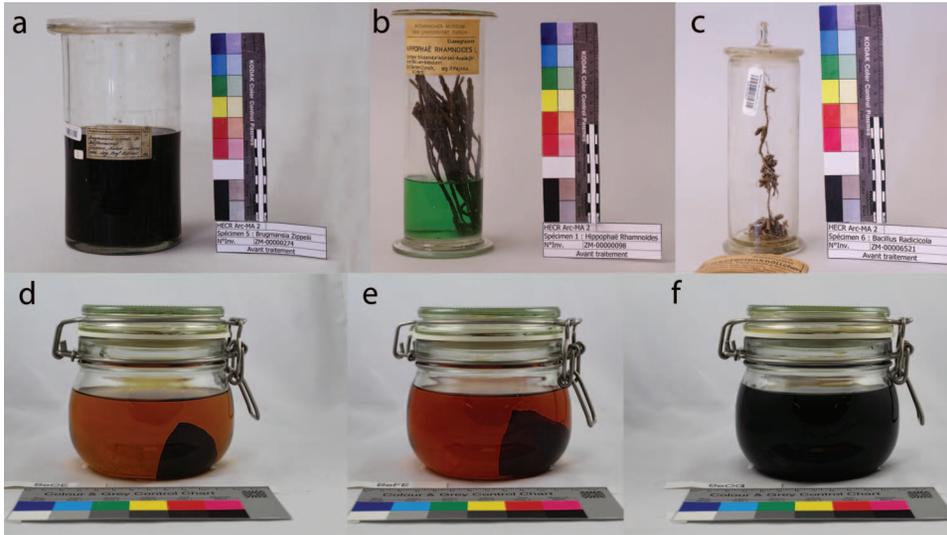


Figure 2. Examples of degradation observed in the fluid collection of the University of Zürich's Botanical Museum: (a) opacity, (b) green color of the liquid, (c) total evaporation and specimen discoloration, (d) beetroot without fixation preserved in ethanol, (e) beetroot fixed in formaldehyde and preserved in ethanol, (f) beetroot without fixation preserved in glycerol (©He-Arc).

liquid, generally brown, completely obscured visibility of the plant (Fig. 2a). In some jars, we detected unnatural colors, such as a flashy green (Fig. 2b). Generally, this kind of green color does not derive from the plant and could be due to copper elements used for mounting the specimen or the addition of copper salts as a preservative agent. However, preliminary analyses performed by X-ray fluorescence spectroscopy did not allow the detection of copper in the fluid, and we were unable to determine the color's origin.

An additional conservation challenge when working with unknown liquids in jars is potential toxicity. Before opening the jar, it is difficult to determine the nature of the preservation fluid unless information is noted on the jar or on the label. For fluid collections, formaldehyde-containing solutions were the most common preservatives used over the past 100 years. Proposed as a miracle solution for fluid preservation at the end of the 19th century (or at least as a cheap option; Simmons 2014), formaldehyde is an acidic solution. It may be buffered with different products to reach a more neutral pH, depending on the final purpose of the product. Formaldehyde is one of the best fixatives for living tissues (Herbin 2013), but it is a toxic product for humans (World Health Organization 2006, Merck 2020). For this reason, when the nature of the preservation liquid is unknown, it is important to use individual and collective protective equipment to perform identification tests. When considering a fluid collection from the conservation-restoration perspective, the health and safety of the operator and of other people are of utmost importance and must be considered.

The third problem with this collection was the structural alteration of the specimens. This problem is generally related to two main causes: fluid evaporation causing the specimen to collapse at the bottom of the container and inadequate mounting assemblies that have deteriorated and caused damage to the specimen.

Inadequate mounting supports, combined with evaporation or fragility of the specimens, were the causes of several incidents of structural degradation, such as sagging or tearing of specimens in the collection. Without replacing or repairing of these supports, the physical integrity of the specimens is compromised. Moreover, aging and deterioration can cause mounting materials such as cotton threads to slacken and become incapable of supporting the specimen. In addition, the successive relocations of the collections certainly have resulted in inappropriate handling and/or transport, which led to structural damage such as breakage or cracks in the containers. A third of the specimens in the collection showed clear structural alteration, most often in the form of loss of elements (leaves, flowers), deformations, or folds.

Physicochemical deterioration of the specimens can occur at different levels, from the macroscopic to the molecular level. It is therefore difficult to estimate the number of specimens impacted without opening the jars. From simple visual observation, loss of color or crystallization was visible on the surface of some specimens.

Overall, for the collection as a whole, the degradation of sealing materials and the evaporation of fluids have led to the drying out of some specimens. Without conservation-restoration intervention, the plant specimens would have dried out and the fluid become more acidic, causing physicochemical degradation of the specimens.

TECHNICAL SOLUTIONS AND TREATMENTS FOR THE UNIVERSITY OF ZÜRICH'S BOTANICAL COLLECTION

The detailed condition report for the botanical fluid collection of the University of Zürich, with the description of the condition of the plant specimens and the extent of deterioration of the jars and contents as described in the previous paragraphs, allowed us to determine which treatment to adopt for affected specimens. The Botanical Museum aimed to improve the mechanical stability of the wet collection to ensure long-term preservation and enhance the aesthetic appearance for future exhibition. It was decided that jar seals should be removed in order to access specimens and fluids in order to assess the state of conservation of the collection.

The different goals of this part of the work were (1) to examine specimen condition in more detail, (2) to identify the fluids present in the jars, and (3) to develop a method to conserve and restore damaged specimens. The second goal was particularly needed to identify the nature of the preservative fluid as either formaldehyde or alcohol and, in the case of alcohol, to estimate its concentration. For some fluids, we were able to carry out complex laboratory analyses (gas chromatography/mass spectrometry data, not shown), but for the whole collection, we carried out spot tests to identify the presence of formaldehyde using Schiff's reagent and alcohol concentration using the Alcomon systemTM (Simmons 2014).

The jars and their lids had to be cleaned to remove adhesive residues and drips due to fluid leaks as well as dust accumulated during storage. Replacement of fluids was carried out to eliminate toxic chemicals, rehydrate specimens, ensure long-term preservation, and restore the transparency of the fluid to allow for examination of the specimen. Consolidation and/or gluing were carried out to repair any breakage and damage of the specimens. The old mountings were repaired or improved, and new mounting systems were created when necessary. Finally, new seals were chosen for long-term sealing of the jars.

The choice of replacement preservative was 70% ethanol because we wanted to eliminate any formaldehyde (due to health risk), prevent mold growth, and maintain only alcohol-preserved specimens in the collection. We implemented the fluid replacement using progressive readaptation/rehydration baths with gradually increasing concentrations of ethanol in

demineralized water, from 0% to 70% ethanol with steps of 10%, 20%, 40%, and 60% (Dangeon 2016). Adaptation baths are necessary to avoid stress on the specimens that could lead to structural deformation. Sudden changes in concentration and the rapid addition of fluid can result in osmotic shock and pH variation, leading to swelling or shrinkage of tissues and rupture by mechanical tension.

If an original solution is not contaminated or soiled, the question may arise as to whether to simply top it off with fluid or to completely replace the original solution. Because the specimen has usually reached an equilibrium with its environment, complete fluid replacement can modify this equilibrium and result in damage. For a few jars in the collection that did not require any intervention other than topping off, we tested the alcohol concentration of these jars and filled them accordingly. If formaldehyde was detected as preservative solution, it was always replaced by ethanol for the previously mentioned safety reasons. For future research, a vial containing part of the initial solution was retained and stored next to the specimen jar with the same labeling as the jar itself.

To carry out repair of damaged specimens, we evaluated different adhesives. This evaluation led us to collodion, a nitrocellulosic adhesive. This adhesive can be adapted according to required interventions. It can be used concentrated for bonding and diluted for consolidation repair.

Mounting assemblies in the collection were typically old systems made up of a glass plate with cotton threads to hold the plant in place. These threads slackened and caused damage to the specimen. We reused the plates by drilling them in several places and passing nylon threads to hold the specimen on the plate.

Internal labels were not originally used in the collection; however, these are necessary to preserve information. We chose to create 5 × 3 cm labels made of alcohol-resistant paper (Resistall™) and written with ink (Sakura Pigma Micron) that is also resistant to water and alcohol (Dubus and Saïd 2011), and these labels were placed inside the jars (Simmons 2014, Dangeon 2016).

For the final sealing step, all flat lids were ground to improve the mechanical grip (Moore 1999). The final seal was chosen based on leak test results using a CO₂ meter. We opted for a neutral silicone (Würth®, Neutral oxime silicone) because it retains elasticity for a future reopening, generating less mechanical stress on the lid.

It is important to stress the usefulness of monitoring the fluid collection. Regular monitoring of alcohol levels should be carried out during long-term storage. The jars of this collection are still kept in the same storage spaces, but we have installed a new ventilation system that allows the humidity to remain between 45% and 60%, monitored using a thermo-hygrometer. The light installed in this room turns on only when a person is present in order to minimize light damage.

THE PROBLEM OF DISCOLORATION OF PLANTS PRESERVED IN FLUID IN BOTANICAL COLLECTIONS: THE FLUIDIS PROJECT

Specimen discoloration becomes problematic when the transfer of pigments from the plant to the fluid leads to the complete opacity of the liquid preservative. In this case, the exhibition value is completely lost, as the specimen resembles a jar full of dark liquid (Fig. 2a). The research value is also partially compromised, as observations of the specimen in the jar are no longer possible. This situation requires, from a conservation point of view, time- and resource- consuming procedures to correct. The jar needs to be opened and the fluid replaced. If the discoloration process is not stopped, the procedure will need to be periodically repeated.

This phenomenon appears to be linked to the presence of specific pigments inside certain plant species (Hendry et al. 1987, Delgado-Vargas et al. 2000). However, the causes of this leaching are, at the present time, still unclear. The pigment leaching might be related to an incorrect fixing procedure at the time of specimen preparation, to the nature of specific plants species, or to a reaction between plant pigments and certain preservative fluids (Moore 2010). The second reason might explain why, in some cases, the phenomenon occurs again after the fluid is replaced.

With the FLUIDIS project, we wanted to understand the mechanisms and phenomena related to the discoloration of botanical specimens preserved in fluid. For this purpose, we selected plant species more prone to discoloration to prepare model samples reproducing conditions found in botanical fluid collections. We tried several fluids in order to determine the influence of fixative and preservative products on plants. The prepared samples were then monitored and analyzed by means of a multianalytical approach including spectroscopic and chromatographic techniques. Particular attention was paid to the detection in the fluid of the pigments responsible for the discoloration phenomena and to the search for the method to fix them in plants to prevent color migration. The preliminary results of the FLUIDIS project related to visual observations and spectroscopic measurements are presented in this article. Characterization of colorants using spectroscopic and chromatographic techniques will be published elsewhere.

MATERIALS AND METHODS

The Plants

Many plants are known for their coloration properties and traditionally used in tincture preparations to dye textiles (Perego 2005); therefore, we assumed these would be more prone to release pigments in the preservation fluids. We selected representative plants with these characteristics, taken from different categories: fruit and roots, flowers, green leaves, and resin producers. We choose to represent fruit and roots specimens with red kohlrabi (*Brassica oleracea*), red chili pepper (*Capsicum annuum*), beetroot (*Beta vulgaris* subsp. *vulgaris*), and fresh and dried walnuts (*Juglans regia*). We used lavender (*Lavandula angustifolia*) to represent flowers, mint (*Mentha suaveolens*) to represent green leaves, and pine branches (*Abies alba*) to represent resin producers. We used fresh plants obtained through a collaboration with the Botanical Garden of the University of Neuchâtel. We started a first set of tests in summer with five plants: red chili pepper, red kohlrabi, lavender, mint, and pine branches. A second set was prepared in autumn with fresh walnut and beetroot. We also performed tests on detached pieces of the flowering parasitic plant *Rafflesia* from the Botanical Collection of the University of Zürich because this specimen exhibits observable discoloration.

The preservative fluids and the fixative solutions.—We wanted to determine the influence of both traditional recipes and modern techniques for fluid preservation. We chose two fixatives: formaldehyde (Merck, 4% buffered formaldehyde solution, pH 6.9) and FAA, a ready-to-use mixture of formaldehyde (4%), ethanol (50%), and acetic acid (5%) (VWR International, FAA histological fixative). We used four preservative solutions: 70% ethanol, rum (37.5% ethanol), 4% formaldehyde, and glycerol (Figure 3). Formaldehyde was used both as a fixative and as a preservative solution.

We choose to use a 70% ethanol solution, made from absolute ethanol (VWR International, ethanol absolut) and demineralized water, as a preservative solution because this is currently a commonly used solution in natural history botanical collections.

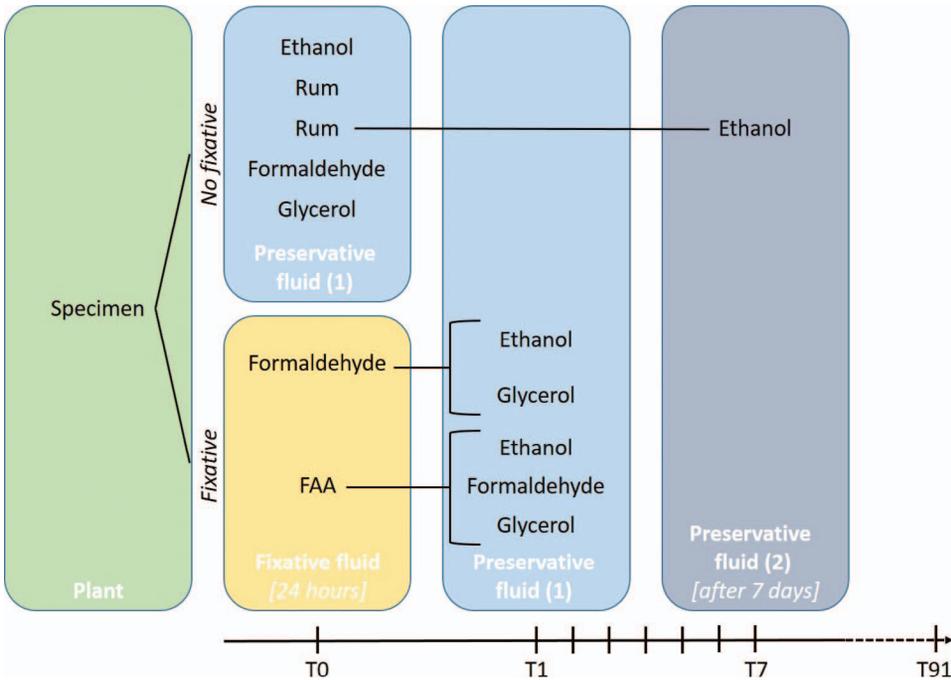


Figure 3. Scheme summarizing the solutions and fixatives used for the FLUIDIS tests as well as the temporal sequence of the experiments (©He-Arc).

We selected white rum because this is a traditional preservative still used by botanical researchers in the field (J. Grant, University of Neuchâtel, pers. comm.). In fact, as fluid preservatives are difficult to transport during scientific expedition abroad, researchers commonly use locally obtained alcohol to preserve freshly-collected plants, and then specimens are transferred into ethanol once back in the university or museum. We used a common commercially available rum brand (Bacardi, 37.5% vol alcohol, white rum) for the project.

We used a ready-to-use 4% formaldehyde solution, buffered with phosphate to protect tissue structures (Merck, formaldehyde solution 4% buffered, pH 6.9). As previously stated, formaldehyde was used in this study both as a fixative and as a preservative solution. This second use is less common. However, in the collection in Zürich and in many fluid collections, specimens have been preserved in formaldehyde for decades.

Glycerol (VWR International, 70% solution in demineralized water) was chosen as the new alternative solution to preserve collections in fluid. Less hazardous than formaldehyde for humans and the environment, it presents an interesting alternative. Glycerol has the advantage of being nontoxic in contrast to formaldehyde or ethanol. Glycerin has been used for a long time, especially for seed collections and for clearing specimens. It has been proposed as an alternative to toxic products for specimens in fluid (Van Dam 2018).

Experimental Methodology

We put specimens in fluid (one or three replicates) following recommendations for the duration of fixation of 24 hours (Simmons 2014) but also leaving some specimens in the fixative, in particular, formaldehyde, during the whole duration of the test, because many

historical collections have specimens that have been fixed and left in fixative over long time periods.

Each specimen was weighed before putting it into the fluid to have an idea of the ratio between the weight of the plant and the quantity of pigments released. The plant was then put into either 250 ml of fixative solution for 24 hours or 350 ml of preservative. After 7 days, the specimens in rum were transferred to 70% ethanol. The kohlrabi and the beetroot were cut into quarters in order to maintain a higher surface-area-to-volume ratio between the specimen and the fluid. For the same reason, the fresh walnuts were cut in half.

Samples of each preservative solution and of each fluid after 3 and 9 months were kept for further analysis (i.e., UV-vis spectroscopy).

We used glass jars of 500 ml (Ikea, model Korken), with a resealable rubber-gasket lid to facilitate regular analyses of the fluid.

Analytical Techniques

Photographic documentation was taken regularly, first at the moment of jar preparation and then after 1, 2, 3, 4, 7, 14, 21, 28, 35, 77, and 91 days in order to monitor the density of coloration of the fluid and the change of color of the specimen. Colorimetric measurements of fluids were taken at the same time of photographic documentation, with a colorimeter X-Rite Ci6x equipped with a stand and cell for liquid measurements. Data were analyzed with Microsoft Excel software for color and luminosity comparison. Delta (Δ) E was calculated, using the colorimetric data of the conservation fluid as reference, according to the following mathematical equation (Zuppiroli and Bussac 2012):

$$\Delta E = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2}$$

where L_1^* , a_1^* , b_1^* are the colorimetric data of the clean conservation fluid and L_2^* , a_2^* , b_2^* are the colorimetric data of the fluid after x time; L^* , a^* , b^* are the coordinates allowing one to represent the color in a space, where L^* is the parameter indicating the lightness of a color, a^* the tonality going from green (negative values) to red (positive values), and b^* the tonality going from blue (negative values) to yellow (positive values); and ΔE is the difference of color between two samples (in this case, the same sample at two different times).

RESULTS

The results of the tests were different depending on the plant species, the fixation step, the process, and the preservation fluid. Only the most representative results by group of plant are described here; all results are summarized in Table 1.

Kohlrabi

Every specimen of red kohlrabi had discolored after 91 days in fluid. The specimen preserved in glycerol, with and without fixation, had deformed and shrunk as if it were dried from the inside. Every preservation fluid gained a pale yellow color, except the jar with the specimen fixed with FAA and preserved in glycerol. This fluid showed no coloration, but the specimen was discolored, indicating that this solution is not suitable for the preservation of a specimen's color.

Table 1. Summary of the results obtained for the FLUIDIS project (ΔF).

Fixative	Conservation solution	Red kohlrabi			Pepper			Mint			Lavender					
		ΔF	Fluid coloration	Fluid color	Specimen aspect	ΔF	Fluid coloration	Fluid color	Specimen aspect	ΔF	Fluid coloration	Fluid color	Specimen aspect			
No fixative	Ethanol	9.49	+	Yellow	\emptyset	21.30	+	Orange-red	\emptyset	22.08	+	Green	\emptyset	9.76	++	Green-yellow
	Rum	1.13	+	Yellow	D	2.66	+	Pale green	\emptyset	5.00	+	Pale green	\emptyset	4.26	+	Pale yellow
	Rum-ethanol	2.96	+	Pale yellow	D	16.36	+	Deep yellow	\emptyset	10.00	++	Green	C	2.89	+	Pale green
	Glycerol	4.84	++	Yellow	S	1.96	+	Pale yellow	\emptyset	3.44	+	Pale yellow	\emptyset	6.04	++	Pale yellow
FAA	Formaldehyde	6.19	++	Yellow	\emptyset	2.60	+	Pale yellow	\emptyset	9.81	+	Yellow	C	9.81	++	Yellow
	Ethanol	2.9	+	Pale yellow	D	29.40	++	Orange-red	\emptyset	18.11	++	Green	\emptyset	6.16	+	Green
	Formaldehyde	3.06	+	Pale yellow	D	1.64	-	No color	\emptyset	3.82	+	Pale yellow	C	4.40	+	Pale yellow
	Glycerol	4.84	+	Pale yellow	\emptyset	1.90	-	No color	S	0.70	-	No color	\emptyset	0.99	+	Pale green
Formaldehyde	Ethanol	5.62	++	Yellow	D	27.40	++	Orange-red	S	13.78	++	Green	C	6.32	+	Green-yellow
	Ethanol	5.2	++	Yellow	S	1.00	-	No color	S	3.60	+	Pale yellow	\emptyset	2.60	+	Pale yellow
	Glycerol	7.59	+	Pine tree	S	44.63	++	Beetroot	S	35.01	++	Walnut (fresh)	\emptyset			
	Glycerol	13.04	+	No color	\emptyset	34.52	++	Deep orange	\emptyset	33.08	++	Dark brown	\emptyset			
FAA	Ethanol	13.04	+	Pale green	D	35.52	++	Brown	\emptyset	34.4	++	Dark brown	\emptyset			
	Rum	13.04	+	Pale green	\emptyset	37.6	++	Deep yellow	\emptyset	30.84	++	Dark brown	S			
	Glycerol	13.04	+	Orange	\emptyset	58.8	++	Dark brown	\emptyset	34.52	++	Dark brown	\emptyset			
	Formaldehyde	13.04	+	No color	\emptyset	49.23	++	Brown	\emptyset	34.52	++	Dark brown	\emptyset			
Formaldehyde	Ethanol	13.04	-	No color	\emptyset	19.56	+	Yellow	D S	31.44	++	Brown-orange	\emptyset			
	Formaldehyde	13.04	-	No color	\emptyset	25.52	+	Orange	D	26.23	++	Brown-orange	\emptyset			
	Glycerol	13.04	-	No color	\emptyset	21.47	+	Yellow	S	35.16	++	Brown-orange	C			
	Ethanol	13.04	+	Pale yellow	\emptyset	35.34	++	Orange	\emptyset	21.10	+	Brown	\emptyset			
Glycerol	13.04	-	No color	\emptyset	34.00	++	Brown	S	21.10	+	Orange-brown	S				

D = discoloration.
 S = shrinkage.
 C = change of color.
 \emptyset = no modification.
 + = coloration intensity.
 ++ = level of coloration intensity.

Red Chili Pepper

For this specific set, in addition to color change of the specimen, structural changes of the fruits were also observed. The specimen of red chili pepper reacted differently depending on the fixative and preservative solution. The specimens preserved in ethanol, with or without fixative, showed a lighter external coloration compared to the beginning of the experiment but still maintained good shape. The ethanol solutions assumed an orange color, with some particles in suspension in the liquid. The red chili pepper preserved in formaldehyde without additional fixation was lighter in color and showed slight shrinkage. The specimen conserved in formaldehyde with FAA as fixative maintained good coloration but had slight shrinkage. The three specimens in glycerol were deformed. The colors were altered, either much brighter than fresh specimens or, in the case of the specimen fixed in formaldehyde and conserved in glycerol, darker in color.

Fresh Walnut

All fresh walnut samples colored the fluid used for preservation. The release of color occurred as soon as the specimen contacted the fluid. The samples fixed with FAA and formaldehyde and preserved in glycerol only released a brown color, and the specimens themselves were still visible in the fluid after 3 months. The samples fixed with FAA and conserved in ethanol or formaldehyde released a dark brown coloration, and the liquid became so dark after 3 months that the specimens were difficult to observe. Fluids of all other samples became black in color, and the specimens inside were no longer visible after 3 months.

Beetroot

The samples of beetroot resulted instantaneously in a pink solution color. The sample preserved in ethanol reacted differently with the fixative: without fixation (Fig. 4a) and with formaldehyde (Fig. 4b) as a fixative, the beetroot released a red color; when fixed for 24 hours in FAA, the color was less intense, and the fluid remained orange after 91 days (not shown). The sample kept in formaldehyde showed a red color, darker when not fixed in FAA. The samples in glycerol, with and without fixation, followed the same trend. Without fixation, the sample in glycerol was no longer visible, and the liquid gained a dark red coloration (Fig. 4c). When fixed in formaldehyde, the fluid became red, and when fixed in FAA, the solution of glycerol was orange. The ΔE for the beetroot samples in different fluids (Fig. 5) tended to stabilize after an initial increase. This indicates that the discoloration occurs soon after the specimen is placed in fluid. The decrease of ΔE at days 2–3 for samples fixed prior to immersion in the preservative fluid is due to the fact that the first two measurements were recorded in the fixation fluid and then the plant was moved in the preservative fluid.

Lavender

All specimens had discolored flowers and a lighter branch color, with the exception of the sample placed in ethanol without fixation and the sample fixed in formaldehyde and then ethanol, which were darker. The specimen fixed with FAA and preserved in glycerol showed less discoloration, with the solution being a very pale yellow. Shape change could not be detected due to the small size of the flowers.

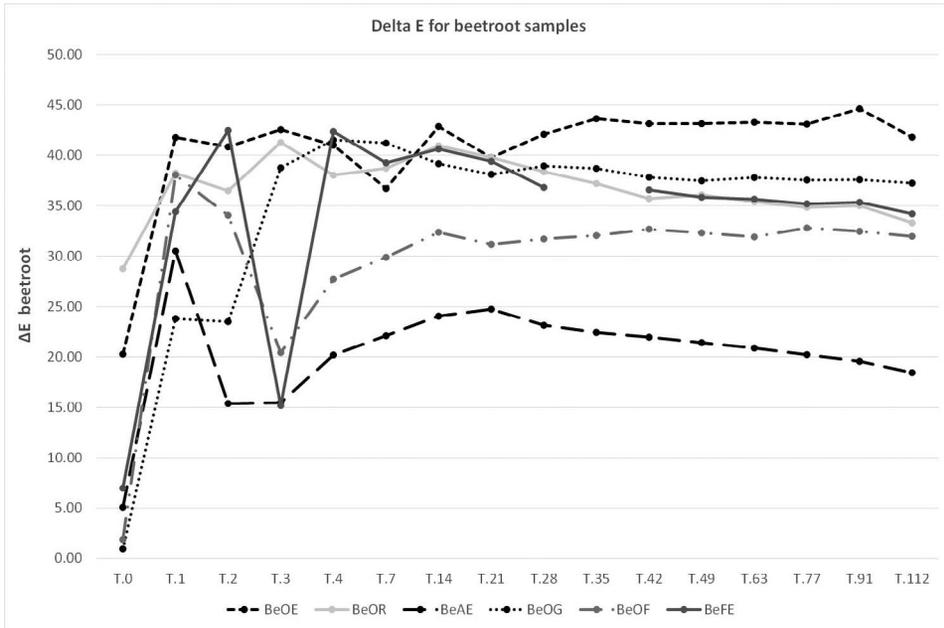


Figure 4. Graph presenting the Delta E variations over time for six beetroot samples (©He-Arc).

Mint

The shape of the mint plant remained stable in each fluid tested. Most of the plants turned from green to brown. Without previous specimen fixation, the ethanol and rum fluids became green, while the glycerol solution tended to turn yellow. The plant changed to a dark brown color in formaldehyde and became almost black in glycerol or when fixed in formaldehyde and preserved in glycerol. The specimen with almost no change was the sample fixed in FAA and preserved in glycerol. This specimen kept a good shape and lost a little color, but the preservative fluid showed no visible coloration as assessed by eye.

Pine Branches

The pine branch samples were less impacted by discoloration of the specimen or of the fluid. There was some pale change in color with specimens in ethanol, FAA-glycerol, and formaldehyde-glycerol. In retrospect, based on our observations during jar preparation, we suspect that the samples may not have been fresh enough to release compounds for our study.

The observations concerning colors reported in the previous paragraphs have been corroborated with colorimetric data. The ΔE values after 3 months, indicating the degree of color change, are summarized in Table 1, together with observations of the specimens and fluids.

CONCLUSIONS

The conservation-restoration work on the fluid collection of University of Zürich's Botanical Museum has given us an insight into the condition of the specimens, jars, and preservatives; the variety of materials used; and the degree of specimen degradation over time and has highlighted the issues involved in the conservation of botanical fluid col-

lections. Often unintentionally neglected, these fluid collections generally require urgent conservation-restoration intervention.

In addition, the conservation-restoration intervention used here has greatly improved the aesthetic value of these scientific and educational objects. It has also given back visual access to the specimens, in turn improving their value for study. Moreover, by documenting and keeping part of the original materials (liquid and seals), we minimized the loss of historical information.

With regard to the important issue of specimen discoloration, the still ongoing FLUIDIS project may help one understand the processes that occur when conserving botanical specimens in fluid. The method developed for monitoring wet specimen discoloration, based on a multitechnique approach, documented color changes, but further evaluation is required for a longer period (>3 months).

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RÉSUMÉ

La Collection botanique de l'Université de Zurich est une collection universitaire constituée entre 1891 et la fin du XX^e siècle (1992 pour le dernier objet inventorié). Les plantes conservées proviennent du monde entier (40 pays) et comprennent toutes les catégories de Plantae existantes (algues, lichens, champignons, plantes supérieures, bactéries). La collection en fluide, tombée dans l'oubli depuis 1976, présente des dégradations importantes, très représentatives des altérations rencontrées sur ce type de collections. Les principaux problèmes sont les fuites des bocaux et le vieillissement des joints. Un autre problème est la décoloration du spécimen dans les fluides. De ce problème est né un projet de recherche intitulé FLUIDIS, qui vise à explorer différentes solutions de conservation et leur impact sur la décoloration des spécimens de plantes. Un travail de conservation-restoration a été réalisé sur les bocaux de la "collection du professeur Ernst." Il a été nécessaire de mettre à niveau le liquide des bocaux de l'ensemble de la collection. La restauration a été effectuée après avoir ouvert les récipients et identifié les fluides. Les spécimens ont été consolidés, réparés, montés si nécessaire, remis de manière progressive dans des solutions d'alcool et enfin scellés. Un protocole d'intervention global a été établi pour le traitement de l'ensemble de la collection. Son application nécessite toutefois une étude minutieuse de chaque spécimen.

LITERATURE CITED

- Cuisin, J. 2016. Conservation des Naturalia: Les collections en fluide—Volet 2. 18–19 February 2016, St-Imier Museum (Switzerland).
- Dangeon, M. 2016. *La collection en fluide du Musée botanique de l'Université de Zürich: Conservation-restoration de six spécimens, identification/sélection de solutions de conservation et tests d'étanchéité des matériaux de scellement.* M.A. Thesis, HE-Arc CR.
- Delgado-Vargas, F., A.R. Jiménez, and O. Paredes-López. 2000. Natural pigments: Carotenoids, anthocyanins, and betalains—Characteristics, biosynthesis, processing, and stability. *Critical Reviews in Food Science and Nutrition* 40:173–289.
- DeWolf, G. P. 1968. Notes on making an herbarium. *Arnoldia* 28: 69–111.
- Dubus, M. and A. Saïd. 2011. Des produits certifiés pour le Marquage des biens culturels. In *Proceedings of the ICOM-CC 16th Triennial Conference*, Lisbon, 19–23 September 2011.
- Hendry, G.A.F., J.D. Houghton, and S.B. Brown. 1987. The degradation of chlorophyll—A biological enigma. *New Phytologist* 107(2):255–302.
- Herbin, M. 2013. *La conservation des collections en fluide*. CeROArt. <https://ceroart.revues.org/3432> (Accessed January 14th 2020).
- Merck. 2020. Safety Data Sheet. Formaldehyde solution 4%, buffered, pH 6.9 (approx. 10% Formalin solution) for histology. Catalogue No. 100496.

- Moore, S. 1999. *Fluid preservation*. Pp. 92–132 in *Care and Conservation of Natural History Collections* (D. Carter and A.K. Walker, eds.). Butterworth-Heinemann, Oxford.
- Moore, S. 2007. Old jar sealants. *NatSCA News* 12:20–22.
- Moore, S. 2010. Preservation of botanical specimens in fluid. *NatSCA News* 19:85–88.
- Perego, F. 2005. *Dictionnaire des matériaux du peintre*. Edition Belin, Paris. 895 pp.
- Simmons, J.E. 2014. *Fluid Preservation: A Comprehensive Reference*. Rowman & Littlefield, Lanham, Maryland. 347 pp.
- Van Dam, A. 2018. Finding a cure for carcinogenic collections. Oral presentation at PFC2018 Conference, Paris, 5–7 December 2018.
- World Health Organization. 2006. Formaldehyde, 2-butoxyethanol and 1-tert-butoxypropan-2-ol. In *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans*, volume 88. International Agency for Research on Cancer, Lyon (FR).
- Zuppiroli, L. and M.-N. Bussac. 2012. *Traité des couleurs*. Presses polytechniques et universitaires romandes. Lausanne. 442 pp.