Chapter 3

New Chemical Insights into the Ancient Molluskan Purple Dyeing Process

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Archaeological and chemical evidence associated with ancient dye vats used for purple dyeings have provided new scientific perceptions regarding the various stages of the process of dyeing with the pigments extracted from *Muricidae* sea snails. These steps were described two millennia ago by Pliny the Elder in his encyclopedic treatise on the natural history known during the Roman Period, and also in a concise description of the dyeing of the related biblical blue-purple *Tekhelet* dye noted three centuries later by the Talmud. A critical re-analysis of Pliny's and the Talmud's writings, combined with the archaeological record and with modern laboratory experiments on all-natural dyeings, have provided new insights into the basic principles of chemistry associated with this craft. The major findings from this current investigation follow: (a) The *"salem"* (Latin for salt) that was mentioned, but not identified, by Pliny as the only external auxiliary reagent needed for purple dyeing must have been an alkaline salt (such as natron, lime, or limestone), which was necessary for the reductive dissolution of the purple pigment. (b) Similarly, the Talmud's mention of *"samanin"* (Aramaic-Hebrew for substances) as an ancillary agent in the *Tekhelet* dyeing process must have also been of an alkaline nature, as in the case of Pliny's salt. (c) Since alkaline conditions were required in the dye bath, seawater, which is naturally slightly alkaline, and not fresh water, could have been used for the initial preparation of the dye bath, with more basic salt added for the necessary higher pH values. (d) A typical

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archaeological vat containing 100 L of dye solution would have contained the meaty flesh of 10,000 *Hexaplex trunculus* sea snails and would produce up to a kilogram and a half of dyed wool – enough for one to two all-purple cloaks or mantles, depending on the depth of color desired. (e) The other two Mediterranean snail species, *Bolinus brandaris* and *Stramonita haemastoma*, produce much less purple pigment than *H. trunculus* and, thus, tens of thousands of these mollusks would have been needed in such a vat; hence these snails would not have been used alone, but rather, when needed, as additions to a *H. trunculus* vat of that size. (f) In order to maintain anaerobic conditions for the slow bacterial reduction of the indigoid pigments to their soluble leuco form, the dye bath would have needed to be covered throughout the process (except for the brief periods of gently stirring the contents of the dye bath), and hence, no significant photo-debromination of the brominated dyes would have occurred as a result of the action of the sun. (g) Consequently, the final general color of the dyeing was dependent on the original color of the raw pigment, whether it was reddish-purple or bluish-purple (violet). (h) Thus, in order to produce the Tyrian or Biblical-*Argaman* red-purple dyeings, a DBI-rich *H. trunculus* species was used alone or with some *B. brandaris* and/or *S. haemastoma* added for even redder dyeings; for the blue-purple dyeings, as in the Biblical-*Tekhelet*, an IND-rich *H. trunculus* was used alone. (i) The direct chemical evidence to date indicates that the production of the molluskan purple colorant solely as a pigment for painting was originated by the Minoans in the Aegean approximately four millennia ago; however, the transformation of the purple pigment into a dye for the dyeing of textiles originated with the Levantine Phoenicians a half millennium later.

Introduction

History and archaeology have recorded that the colors of the garments and palatial furnishings of royalty, military generals, and high priests were various shades of purple. These include red-purples similar to the common names of bordeaux, maroon, burgundy, etc., as well as blue-purple or violet colors. In antiquity, these purple dyeings were performed along the Mediterranean basin on cleansed woolen fleeces by using the pigments extracted from various sea snails, *Muricidae* mollusks, primarily from the *Hexaplex trunculus* (also known as *Murex trunculus*) species, *Bolinus brandaris*, and *Stramonita haemastoma* species (*1*), as depicted in Figure 1. It has been established that the latter two species can only produce red-purple dyeings, whereas there are two chromatic varieties of *H. trunculus* species, perhaps even zoological sub-species; one that can produce the red-purple dyeings similar to that produced from *B. brandaris* and *S. haemastoma* snails, and the other that produces blue-purple or violet dyeings (*2*, *3*). A review of the history and chemistry of these molluskan dyes (*4*, *5*), and the analytical methods developed for multi-component identifications of *Muricidae* pigments via liquid chromatography have been previously published (*2*, *6*). The main components of molluskan pigments are the indigoids, but they could also contain minor contributions from isatinoids and indirubinoids, and these have all been discussed at length elsewhere (*2*, *3*, *6*).

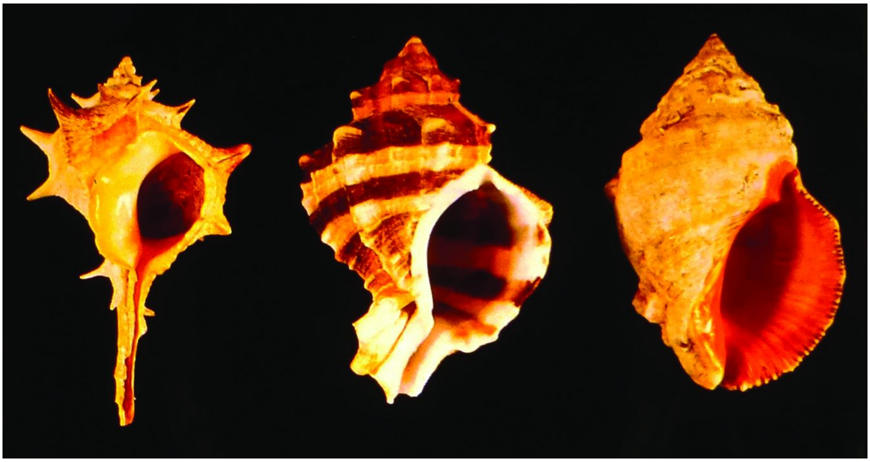

Figure 1. The three most common purple-producing Muricidae sea snails inhabiting the Mediterranean (from left to right): Bolinus brandaris, Hexaplex trunculus (also commonly known as Murex trunculus), and Stramonita haemastoma. (Courtesy of the Eretz-Israel Museum, Tel-Aviv.)

The most detailed ancient account of the various steps associated with purple dyeing from molluskan snails was provided by the 1st century CE Roman historian, Pliny the Elder (Gaius Plinius Secundus), in his epic work titled *Naturalis Historia* – Natural History (*7*). The excerpts from Pliny's work given in the sections below are based on the Latin version and English translation given by Rackham, with certain etymological emendations offered by this author. There have been various English translations of Pliny's work and these have shown differences in textual styles as well as sometimes ambiguous renditions of the Latin weight and volume measures, and hence the excerpts below do not include these quantities. However, though these translational differences exist, nevertheless, the overall renditions of Pliny's descriptions are all similar.

The main questions that have perturbed historians of Pliny's writings are whether his descriptions of various technologies, and specifically, of the processes associated with purple dyeing, are incomplete or even incorrect, and whether Pliny actually observed the purple dyeing process (*8*). As far as Pliny's working method is concerned, in the Preface of his 37-volume Natural History treatise dedicated to his friend Titus Vespasian Caesar, Pliny specifically states that he was occupied with many official tasks, but yet his work includes 20,000 topics garnered from 2,000 books of 100 authors (*9*). His nephew, Pliny the Younger, concurs and writes in his letter to Baebius Macer (*10*, *11*) how extremely busy his uncle was, but he still found the time to write multi-volume works. Thus, there is no doubt that probably most of what Pliny wrote about was gleaned from other sources. However, Pliny also clearly states that he introduces new ideas not written anywhere (*9*).

> "… and to these I have made considerable additions of things, which were either not known to my predecessors, or which have been lately discovered."

Hence, not every topic that he discusses in his Natural History is one that has been copied or re-written from other sources. For some of his descriptions of chemical processes, he probably was an eyewitness. The author (or authors) of the relevant Wikipedia articles indicate that Pliny's description in Book 33 of the gold mining process is so detailed that it was probably written as an eyewitness account, and based on the proximity of where Pliny was stationed he could have certainly visited the mine sites (*12*, *13*). So, it may have been possible that he also witnessed the purple dyeing process.

However, the question of whether Pliny actually saw the technology first-hand or was told about it or read it from other sources available to him at the time is an irrelevant matter. The ultimate point is: was Pliny's account correct?

From the various experiments conducted by this author on purple pigments over the last two decades, certain insights into the dyeing process practiced in antiquity have now been gained. These investigations include optimizations of all-natural purple dyeings produced from the *H. trunculus* molluskan pigments (*14*). These studies have shown that Pliny's descriptions parallel that of modern laboratory experiments performed in reconstructing natural dyeing with molluskan purple pigments.

The Preliminary Stages: From Snail Collection to Pigment Production

Collecting Live Snails

According to Pliny:

> People strive to catch this shellfish alive, because it discharges this juice with its life.

Personal experience has shown that *Muricidae* sea snails, especially *H. trunculus* species, can be found in relatively shallow waters (ca. 1 m) in rocky shore areas full of seaweed vegetation, such as depicted in Figure 2. These snails are usually partly concealed as they are found burrowed in the sandy seabed with only a small hump from their shell visible. They must be collected live – and kept alive until the pre-dyeing stage is begun – in order to be effectively exploited for the production of the purple pigment. This is due to the necessity of avoiding the premature ejection of the purple pigment from the dying animal. As the snail expires, it expels the purple pigment, and if these snails are still in the water, then the pigment will be lost as it is dispersed in the sea.

Figure 2. The coastline of the beach at Akhziv in modern northern Israel where Muricidae sea snails inhabit the seabed of this rocky and flora-filled seascape.

Cracking the Shell To Expose the Chromogenic Gland

Pliny writes:

> The murex … has the famous 'flower of purple', sought after for dyeing robes, in the middle of its throat: here there is a white 'vein' of very scanty fluid from which that precious dye, suffused with a dark rose color, is drained, but the rest of the body produces nothing.

The exposed hypobranchial glands of two *H. trunculus* snails are shown in Figure 3, and in one snail the gland (Pliny's "vein", the "flower of purple") is indeed white, and in the other snail it is gray-colored, and yet in others it could be beige. The gland contains the colorless brominated and unbrominated indoxyl precursors in its fluid (*4*, *5*). As long as the snail is alive, only these colorless precursors exist, and no purple pigment is yet present or produced in the gland. In order to initiate the production of the purple pigment from these precursors, which can be straightforwardly accomplished, the snail shell is strategically cracked so that the sharp broken shell pieces will deliberately rupture the gland. This piercing would then allow the purpurase enzyme, which is naturally present in the gland but physically separated from the precursors, to come in contact with the chromogens. This interaction initiates the necessary spontaneous enzymatic hydrolysis steps on the precursors and a series of natural photochemical air-oxidation steps follow. This procedure is performed without heating in order not to destroy the required enzyme. In a matter of a few days, the "dark rose color" of the pigment is produced (also see below). In modern times, *Muricidae* snails are sold as seafood, *fruits de mer* and escargot, at various markets in Mediterranean countries and cooking them in boiling water destroys the enzyme and thus the purple pigment is not produced.

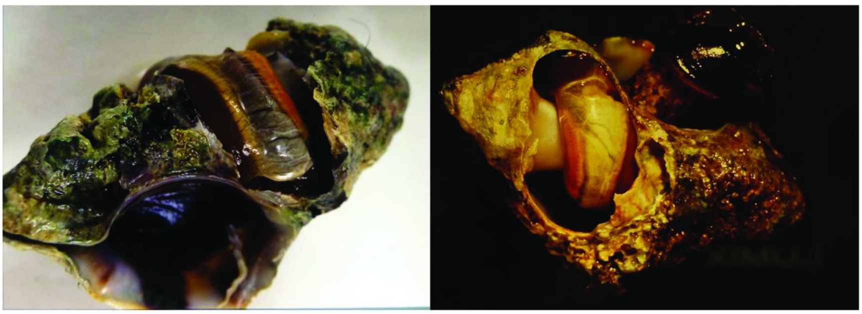

Figure 3. Two Hexaplex trunculus mollusks with their shells cracked open to expose their hypobranchial glands: (left) gray vein, (right) off-white vein.

Separating the Meaty Snail from the Shell

According to Pliny:

> From the larger purples they get the juice by stripping off the shell, but they crush the smaller ones alive with the shell, as that is the only way to make them disgorge the juice.

Once the shell has been cracked and the gland exposed or has been punctured, then the snail meat with the developing pigment can be forcibly separated from the shell. The shell is discarded as it will occupy too much space in the vat and the entire snail meat – with the developing pigment adhering to it – is placed in a vat. This snail meat is a necessary nutrient for the reductive bacteria also present in the snail (as described below). With smaller snails, the act of stripping off the snail meat from its shell is more difficult and unnecessary; the simple act of crushing the snail shells insures that the gland has been punctured and that the development of the purple pigment has begun. The crushed smaller snails are added to the vat containing the flesh of the larger snails.

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Further Pigment Development

From Pliny's treatise:

> Subsequently the vein of which we spoke is removed, to this, salt [*"salem"* in Pliny's Latin] has to be added …; three days is the proper time for it to be steeped, as certainly the fresher it [the extract] is the much stronger it is.

Experiments have indeed shown (*14*) that the visceral mass of snail flesh with the pigment adhering to it needs to be left undisturbed for a total of about 3 days from the time it was initially produced by the rupturing of the gland until the purple or violet pigment is spontaneously fully developed.

Fermentative Anaerobic Bacterial Reduction

A necessary step for any dyeing – natural or synthetic – is that the dye be solubilized and thus can form strong chemical and physical bonds to the textile fibers on a molecular level. Since the main indigoid components of the molluskan purple pigment are relatively insoluble in aqueous solutions, they must first be converted to a soluble form. This water-soluble state is the reduced indigoid molecule, which is yellow and thus much less-colored or "whiter" than the original highly colored indigoid, and hence this reduced form is known as the "leuco" state. Figure 4 shows the schematics of the reduction of an indigoid to its leuco, moderately soluble non-ionic acid form, and further ionization.

Though 2,000 years ago Pliny obviously never identified the biochemical mechanism or the reducing agent necessary to solubilize the indigoids, in antiquity the reducing agent for such a process must have been the bacteria present in the snail meat. Bacteria found in various plant fermentation vats were successful in the reduction and dissolution of the indigo pigment from plant sources. For example, an anaerobic moderate thermophilic bacterium, named *Clostridium isatidis*, capable of reducing indigo was extracted from a fermented medieval woad (*Isatis tinctoria* L.) vat (*15–17*). It was found that growth occurred at pH 5.9 – 9.9 (initial pH) with an optimum at 50 °C of pH 7.2 ± 0.2 (constant pH). Further, at pH 7.8, the temperature range for growth was 30 – 55 °C with the optimum at 49 – 52 °C.

The mechanism by which the bacterium *C. isatidis* reduces indigo was not initially understood. However, it is believed to be a direct interaction between the bacteria and indigo and not as a result of the metabolic products, though H_2 gas has been detected as a product of the fermentation; CO_2 gas and acidic products have also been identified (*18*, *19*). Electrochemical measurements showed that the mechanism of the bacterial reduction of indigo appears to be due to two features: the bacteria produce extracellular factors that decrease indigo particle size, and they also generate a negative potential of sufficient magnitude. The solid state bacterial-driven reduction for indigo dyeing in the absence of a redox mediator requires direct contact between bacteria and the solid indigo and

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transfers an electron from the bacterial cell interior to a solid external electron acceptor (indigo).

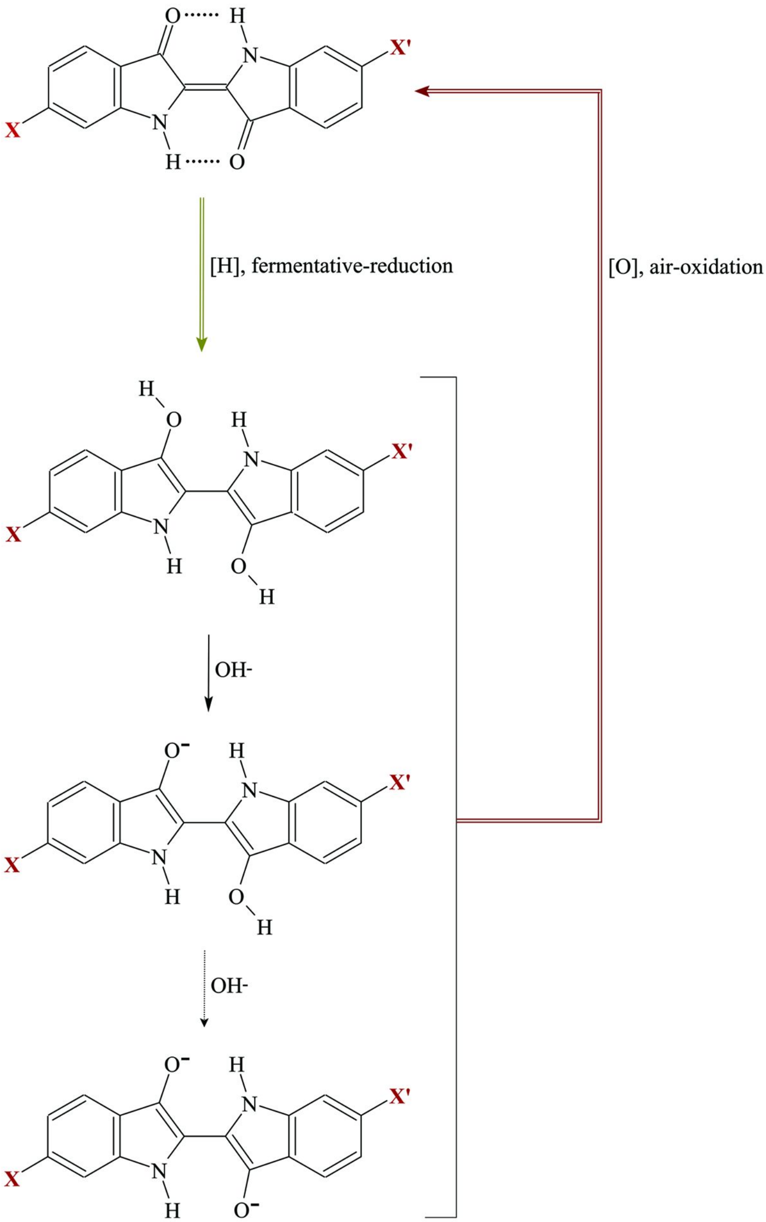

Figure 4. Schematic of the reduction of the indigoid components from the purple pigment and re-oxidation to the original pigment: indigo (IND) for X = X′ = H; 6-bromoindigo (also known as monobromoindigo, MBI) for X = Br, X′ = H; 6,6′-dibromoindigo (DBI) for X = X′ = Br.

Other bacterial strains, different from *C. isatidis*, able to reduce indigo have also been found in other fermentation vats, as for example from the Asian indigo-producing *Polygonum tinctorium* plant species (*20*).

Thus, in the case of the molluskan purple pigment, in which the main constituents are indigoids – indigo and its brominated derivatives – similar anaerobic moderately thermophilic bacteria must also be responsible for the reductive dissolution of these pigments, though these strains have not yet been identified. Successful fermentation vats have been produced for all-natural dyeings with molluskan pigments in the United Kingdom, France, and Israel (*14*). In modern times, there are strong synthetic reducing agents, such as sodium dithionite (also known as sodium hydrosulfite), $Na_2S_2O_4$, that can nearly instantly reduce any indigoid. However, in antiquity, since the molluskan pigment was naturally reduced by the anaerobic bacterium acting as a mild reducing agent, the time necessary for the full reduction was a matter of days.

The anaerobic nature of the bacteria can be deduced from the archaeological evidence associated with molluskan purple dyeing. Figure 5 shows a potsherd from a 7th century BCE clay vat used for purple dyeing and excavated from a Phoenician site in Tel Kabri in modern northern Israel. The residual purple pigment is still clearly visible after more than 2,500 years as a result of the undesired oxidation – and precipitation – of the dissolved purple. This pigment has been previously analyzed and shown to contain the three indigoids, which indicates that it is from a molluskan source (*21*). Since this fragment has a curved finished top it is clearly from the uppermost part of the vessel. Thus, the location of this pigment at the very top of the interior of the vat indicates that the liquid contained in the vessel reached nearly the top of the vat. A similar potsherd with residual purple pigmentation at the top was also found at the 8th – 9th centuries Tel Shikmona site near Haifa in modern northern Israel. In order to reduce the amount of atmospheric oxygen from entering the bath – and thus to prevent the unwanted oxidation of the reduced indigoids – the vat would have also been covered with either a slab of stone or wood. Thus, only a small amount of space would have existed between the top of the liquid and the vat's cover. With the various gases produced from the fermentation process, only a small quantity of air would have been present in the head space above the liquid. This would aid the anaerobic nature of the bacterial action. The lid of the vat would have been opened for very brief periods in order to stir and mix the contents of the liquid very gently so as not to introduce much air into the dye bath, but, except for these very short interludes, the cover would have stayed in place to prevent the entrance of air into the dye liquid. This preventative measure has filtered down through the ages so that already two centuries ago, historic dyeing books directed dyers to fill an indigo vat nearly to the top and to keep it covered (*22*).

The necessity of keeping the dye bath covered would also have prevented sunlight from entering the dye solution. Hence, no significant photo-debromination of the brominated indigoids would have occurred, and the compositions of the dyes in the woolen dyeing would have reflected the constitution of the original pigment. Different dyes have different affinities to textile fiber materials and since the purple pigment consists of a number of colorants, the compositions of the dyes in the dyed fibers will, in general, be different from their compositions in the original pigment. Nevertheless, the overall color of the dyeing is reflected by and dependent on the original color of the raw pigment, whether it is reddish-purple or bluish-purple (violet). Only the *H. trunculus* sea snail species produces the violet singly-brominated MBI dye in significant quantities. This snail's pigments also contain the other two indigoids – the doubly-brominated DBI dye and the unbrominated indigo (IND) dye – in varying quantities. The *H. trunculus* mollusks consist of two chromatic varieties: one produces red-purple pigments owing to its relatively large quantity of DBI, whereas the other produces bluer, violet, pigments due to its relatively abundant IND dye (*2*, *3*). Thus, in order to produce the Tyrian or Biblical-*Argaman* red-purple dyeings, a DBI-rich *H. trunculus* species was used alone or with some *B. brandaris* and/or *S. haemastoma* added for even redder dyeings; for the blue-purple dyeings, as in the Biblical-*Tekhelet*, an IND-rich *H. trunculus* was used alone.

Figure 5. The interior of a purple-stained potsherd, part of the upper mouth of a dye vat, excavated at 7th century BCE Tel Kabri in northern Israel. (Courtesy of the Tel Kabri Expedition, Tel-Aviv University.)

In order not to destroy this moderately thermophilic micro-organism, the dye bath was not boiled, but raised to a moderately hot temperature. This is in accordance with Pliny's statement whereby he describes the heating from a heat source that is not directly in contact with the dye bath, as follows:

> It [the snail flesh with the adhering pigment] should be heated … with … water … and kept at a uniform and moderate temperature by a pipe brought from a furnace some way off. This will cause it gradually to deposit the portions of flesh that are bound to have adhered to the veins.

Pliny's lyrical description of the application of indirect heat to the dye bath can be evident from the presence of the charred areas, visible even today, on the exterior of the more than 2,500 year-old potsherd (Figure 6), which is the reverse side of the fragment from Figure 5. This marking would be the result of heating the vat to moderate temperatures by means of, for example, hot charcoal. The scenario could have been that the vat would have been placed in a pit in order to maintain relatively constant warm to hot temperatures while placing smoldering wood pieces all around the vat. These chunks could have easily been removed as they cooled down and replaced by fresh hot charcoals as needed. A comparable practice to this ancient method of heating can be seen two centuries ago by the placement of hot embers around the vat (*23*).

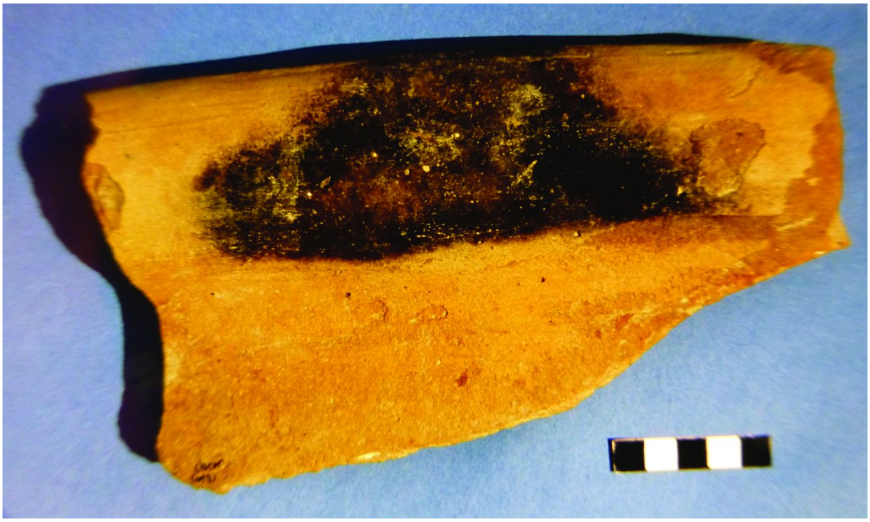

Figure 6. The exterior of the potsherd from Figure 5 with visible charred stains. (Courtesy of the Tel Kabri Expedition, Tel-Aviv University.)

Pliny's Salt

Pliny indicated that a salt (*"salem"*) was a necessary auxiliary agent in the purple dyeing process. The misconception of many interpreters of this statement was that Pliny referred to ordinary salt, i. e., sodium chloride (NaCl). However, experiments have shown that this neutral salt has no effect on the necessary dissolution of the indigoids and plays no part in the reduction. However, from the general chemistry topic of acids and bases, students are taught that there are acidic and basic salts too. It is then obvious that while Pliny was not of course discussing the chemistry principles associated with the reductive dissolution of the indigoids, his mention of a salt must have alluded to a basic salt for the reasons discussed below.

The solubility of a reduced leuco-indigoid increases with increasing pH. Figure 4 shows that an increase in alkalinity generates the soluble mono-anionic leuco from the moderately soluble non-ionic acid, and a further increase in the pH would then produce the soluble di-anion. The relative amounts of these leuco-species were studied for indigo with the following results (*24*): At pH 8,

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about 80% exists as the acid and 20% as the mono-anion; At pH 9, about 60% is the acid, almost 40% mono-anion, and less than 5% di-anion.

The mono-anion reaches a maximum of about 70% at a pH of about 11, with nearly equal amounts of the acid and di-anion also present. However, since wool was to be dyed, the pH must be less than 9 in order not to cause damage to the proteinic textile.

Though that work was produced for indigo itself, it may be assumed that similar results would be obtained for brominated indigoids – MBI and DBI (see Figure 4) – originating from molluskan pigments. Hence, dissolution of the indigoids in their reduced leuco state would be increased in an alkaline environment; however, in the limiting pH needed for dyeing wool, the majority of the reduced leuco-indigoids would be in their nonionic form with some mono-anion also present. Such an alkaline system can be naturally produced by various means and are hereby discussed.

Stale Urine

The use of stale urine, which contains ammonia from the decomposition of urea caused by bacterial contamination, can produce moderately alkaline solutions of about pH 8 (*25*, *26*). Stale urine was a popular reagent in Europe in the 18th and 19th centuries for the dyeing of indigo (*27*).

However, during the fermentation process acidic compounds are generated and reduce the initial pH of the dye mixture to acidic levels and will decrease the solubilities of the brominated leuco indigoids. Thus, the alkalinity of the dye bath would have needed to be monitored in antiquity, not a simple process unless the dyer was willing to use the sense of taste and/or touch on a portion of the bath in order to determine its pH. With this lowering of the pH of the dye bath, it was necessary to add more of the base on a regular basis until fermentation ceases. If stale urine was to be used as the alkaline medium, then a considerable quantity of it would be necessary in order to raise the pH of the dye bath to the desired degree of basicity, an action that will considerably dilute the dye bath to an unacceptable level as it would produce inferior purple dyeings. Hence, most probably stale urine solutions were not used for producing an alkaline dye bath with the molluskan purple pigment. In addition, of course, this liquid reagent could not have been Pliny's salt.

Soluble Carbonates: Natron or Soda Ash and Potash

A soluble carbonate, such as sodium carbonate (Na_2CO_3) or potassium carbonate (K_2CO_3), can produce strongly alkaline aqueous solutions. Sodium carbonate can be naturally obtained from the raw mineral natron, which also contains some of the moderately alkaline sodium bicarbonate ($NaHCO_3$), or from the ashes of certain plants ("soda ash"), and similarly potassium carbonate is present in wood ash ("potash"). The benefits of these soluble carbonates is that they can be added as solids and thus not dilute the original dye bath, although the level of alkalinity was still needed to be monitored and the carbonate added as needed throughout the reduction process.

Lime

Quicklime, burnt lime, or more simply lime is calcium oxide (CaO), which can be produced from limestone, calcium carbonate ($CaCO_3$), by heating, and when added to the aqueous dye solution generates the alkaline limewater (also known as slaked lime), aqueous calcium hydroxide, $Ca(OH)_2$*(aq)*. The pH values that can be attained are higher than 11 (*28*). Here too, the lime is added as a solid to the dye bath, and, as in the case of the soluble carbonates, will also not dilute the dye bath when added to increase the pH of the dye solution as needed throughout the reduction.

Lime + Soluble Carbonate

A strongly alkaline solution can be produced from the combination of lime with a soluble carbonate, such as the ones described above. For example, with potash, this mixture in water will produce the strong base, potassium hydroxide, according to the following reaction:

$$CaO(s) + K_2CO_3(s) + H_2O(l) \rightarrow CaCO_3(s) + 2\ KOH(aq)$$

The disadvantages of this alkaline combination is that, as with the other salts mentioned above, it could produce pH values that are much higher than 9. Thus, its pH would have also been needed to be monitored as in the above cases.

Limestone

In Pliny's account the only missing ingredient from the process is an alkaline reagent, and any of the above-mentioned salts – natron or soda ash, potash, lime with or without ash – could be possible candidates for Pliny's salt. However, these solids all possess practical disadvantages in this process. These soluble salts can initially produce relatively high pH values, but since acidic products are formed during the fermentation process, the pH of the solution is drastically reduced. For example, in recent natural dyeing experiments (*14*), an initial pH of 9 was produced in the dye bath by means of sodium carbonate just prior to fermentation, and in less than a day, the pH was reduced to an acidic pH of 6.7. In order to solubilize as many leuco-indigoid species as possible (see Figure 4), the pH was then re-adjusted to 9 by the addition of more salt. This repeated cycle of checking the pH and the subsequent addition of more salt lasted for about three days, until the fermentation ceased and no more acidic products were produced, and thus the pH did not change.

A much simpler method was possible in antiquity for maintaining moderate alkalinity with an eye towards the final dyeing step in this time-consuming multi-stage procedure, and it could have been the one used by the ancient dyer. This step invokes the basic principles of general chemistry, even as taught at the freshman university level, and it pertains to the equilibrium constant associated with relatively insoluble salts, the solubility product constant K_{sp}. Thus, use was made of the widely available mineral limestone or chalk (a softer form), either the calcite or aragonite crystalline forms, which is essentially calcium carbonate ($CaCO_3$). This choice of salt was deliberately made due to the fact that unlike

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the other salts mentioned-above, limestone is only a sparingly soluble salt. Its reported literature value for its solubility product constant (K_{sp}) at 25 °C shows a range of values, from 1 x 10^{-8} (*28*), 3.36 x 10^{-9} (*29*), 3.8 x 10^{-9} (*30*), 4.95 x 10^{-9} (*31*), and 8.7 x 10^{-9} (*32*).

Similarly, variable values are given in the literature for the solubility of this salt. For example, from the 66th edition of the CRC Handbook (*31*), the solubilities of the two crystalline forms of calcium carbonate in 100 cc of water are given as follows. Aragonite: 1.53 mg at 25 °C, 1.90 mg at 75 °C; Calcite: 1.4 mg at 25 °C, 1.8 mg at 75 °C.

However, from the 93rd edition of the 2013 online edition of CRC (*29*), the solubilities at 20 °C of these crystalline forms are equal at 0.66 mg per 100 g water. Further, in 100 cc of water, Wikipedia (*32*) reports a solubility of 4.7 mg, and additionally a value of 1.3 mg has been reported (*33*).

In order to evaluate the expected pH of saturated $CaCO_3$ solutions, various aqueous equilibria associated with $CaCO_3$, HCO_3^-, CO_3^{2-}, and the partial pressure CO_2(*g*) above the solution and dissolved in the dye bath (Henry's Law) need to be considered. Some of the pH values reported in the literature for saturated aqueous solutions of calcium carbonate at 25 °C are 8.6 (*34*), 9.95 in CO_2-free water and 8.31 for the normal 0.03 % contribution of CO_2 in atmospheric air (*35*), and 8.27 (*32*). Hence, these values are all in relative agreement that for an aqueous saturated calcium carbonate solution at room temperature and at the normal atmospheric contribution of CO_2, the pH is about 8.3.

The ancients could have then used an excess of this very sparingly soluble salt, with the added value that there would be no need to replenish it during the overall dyeing process, which is not the case with all of the above-mentioned solids. An excess quantity of limestone in general chemistry terms would cause the acids produced in the fermentation process to be the limiting reagents and thus as there would always exist a saturated solution of calcium carbonate, its concentration would be constant even as it neutralizes the produced acids. Even as the action of an acid on the calcium carbonate produces its soluble calcium bicarbonate conjugate, the latter is still alkaline and a buffer system of carbonate/bicarbonate would be established to maintain the necessary alkalinity of the solution. Thus, a constant alkaline environment would be maintained throughout the fermentation and would aid in the solubility of the leuco species, especially the mono-anion, while, at the same time insuring that the pH would be less than 9 so as not to damage the proteinic wool during the later dyeing process. This dual action of the limestone is a double advantage over the other salts.

Archaeological evidence also supports the supposition that limestone may have been used as the salt mentioned in Pliny's description. Small pebble-sized stone pieces were found in a 6th century BCE Phoenician purple dyeing installation at Tel Dor in modern north-central Israel. One of these is portrayed in Figure 7, and it is stained with a soiled dark residue. The original color of this pigment was not visually discerned with the naked eye, but a spectrophotometric analysis of a DMF extract of this residue showed it to be from a molluskan pigment source (*36*). The highly magnified picture now seen in Figure 7 does show that the residue is purple in color. These stone pieces were analyzed by this author in order to determine whether they are, in fact, essentially limestone ($CaCO_3$), or of a different material, as for example lime (CaO). The simplest chemical examination towards this end is to treat the stone sample with a drop of dilute hydrochloric acid, HCl(*aq*). This drop caused an immediate acid-base reaction with frothing, which is clearly a result of the production of gaseous carbon dioxide (CO_2) from a carbonate. This limestone sample, with some of the purple pigment still adhering to it after 2,500 years, may have been used for the production of an alkaline environment at the Tel Dor dyeing site. Though it has been previously conjectured that the role of these limestone pieces was to provide the necessary alkalinity (*36*), the reasons why this compound was specifically chosen over other possibilities has never been clearly explained.

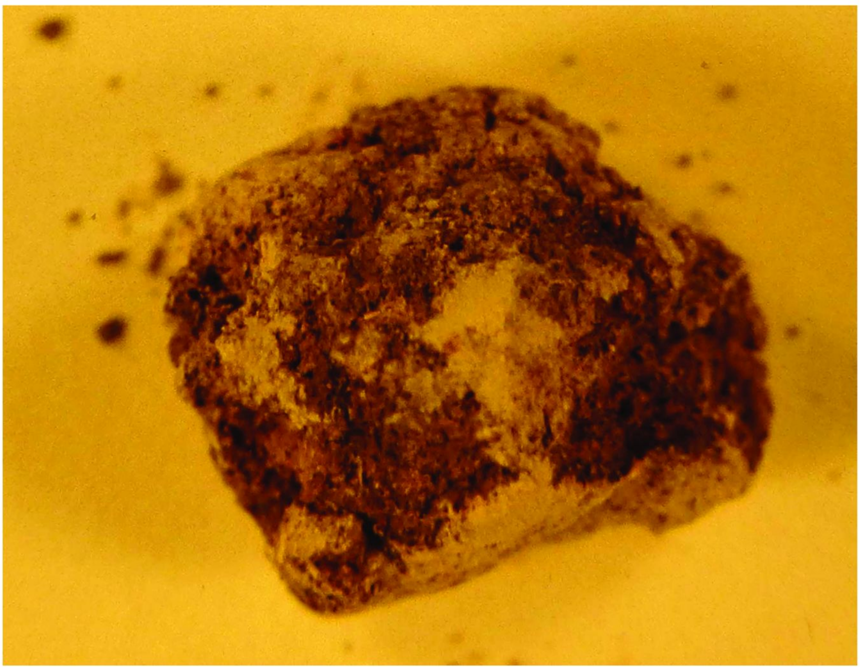

Figure 7. A dark soiled residue on a small piece of limestone found in the channel between two pits at a Phoenician site dated to the 6th century BCE at Tel Dor, in north-central Israel.

The realization that the quantity of the limestone introduced at the beginning of the process decreases throughout the reduction would have been apparent to the ancient dyers as they inspected the vat after the dye bath was emptied. These ancient empirical chemists must have been cognizant of the fact that certain compounds – today we call them acids – can react with the limestone. In fact, legend records that this elementary chemistry principle was already known several centuries before Pliny. The Roman historian, Titus Livius Patavinus, known simply as Livy in English, living 2,000 years ago wrote an epic history of Rome called *Ab Urbe Condita Libri* ("Books from the Foundation of the City"), which covered the periods from the 8th century BCE until his present time. Livy recounts (*37*) how the famous Hannibal, the Punic Carthagian military commander, crossed the Alps in his march into northern Italy in the latter part of the 3rd century BCE. The only way for Hannibal's army to advance was across the rock face. In order to build a more manageable track for the baggage animals and war elephants to navigate down the mountainous slope, the soldiers needed to soften the limestone rocks so that it would then be easier to break them. According to Livy, this softening reaction was accomplished by pouring sour wine vinegar – a weak acid – into the cracks of the limestone.

Though the shells of sea snails are mostly calcium carbonate, they cannot be used to produce the necessary alkaline environment in the dye bath. A molluskan shell is a natural composite biomaterial with superior mechanical properties (*38*). Its complex organo-mineral structure is composed of an organic matrix, which consists of a mixture of glycoproteins and polysaccharides, bound to a mineral phase. This close association produces the resistance of shells not only to fracture but also provides for the resistance of the calcium carbonate to dissolution in water. Thus, shells that are thousands of years old have survived.

Source and Quantity of Water and Mollusks Used for the Dye Bath

Most archaeological dyeing installations for purple dyeing have been found near the coast as this was obviously a normal strategic location for processing the collected sea snails from the nearby waters. Hence, the question that is posed is whether seawater was used to prepare the purple dye bath in antiquity or whether use was made of fresh waters from lakes, streams, wells, etc.

The normal present day seawaters are mildly alkaline with a typical range of pH values of 7.5 – 8.5 (*39*, *40*). At these values, the HCO_3^- ion predominates. When CO_2 from the atmosphere reacts with seawater, it immediately forms carbonic acid (H_2CO_3), which in itself is unstable. This further dissociates to form bicarbonate and carbonate ions. These ions are responsible for the buffering capacity of seawater, and thus seawaters can resist drastic pH changes even after the addition of weak bases and acids. Thus, since an alkaline environment was needed anyway for the dye vat, the ancients definitely could have initially used seawater – and would not have needed to use fresh water – before adding more of the alkaline substance to the bath in order to raise it to the proper alkalinity.

The quantity of liquid needed to fill a purple dye vat can be inferred, for example, from Figure 8, a partially reconstructed dye vat using the original potsherds. Amazingly, this 11th century BCE vat from Tel Keisan in modern northern Israel still shows residual purple stains that have survived 3,000 years (Figure 9). This type of vat would have contained a liquid volume on the order of about 100 L (or more). Previous natural dyeings have shown (*14*) that good depth of color was obtained by dyeing a woolen fleece according to the following ratio:
1 g wool : 7 medium *H. trunculus* snails : 70 mL dye solution

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Figure 8. A partially reconstructed dye vat from an 11th century BCE Phoenician site at Tel Keisan in northern Israel.

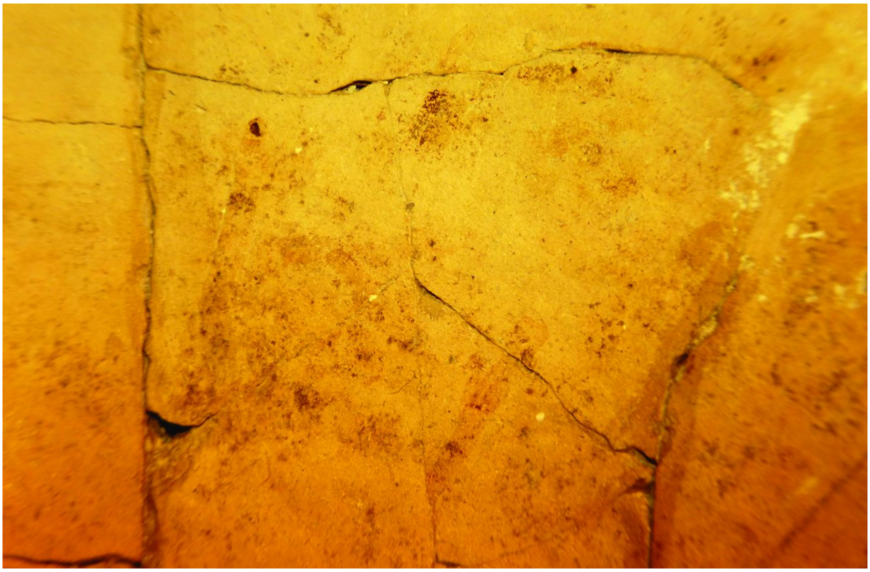

Figure 9. Partial view of the interior of the potsherd from Figure 8 showing spots with purple pigment.

Thus, for a vat containing 100 L of liquid, the flesh of ten thousand *H. trunculus* snails would be needed, and such a vat could dye about a kilogram and a half of wool, which may be enough to produce one or two all-purple royal cloaks or mantles, depending on the depth of color desired.

The other two sea snail species that have been associated with purple dyeing, *B. brandaris* and *S. haemastoma*, produce much less pigment than even the small quantities extracted from the *H. trunculus* snail. Hence, it is a staggering thought to propose that whole dyeings would have occurred in such dye vats with either *B. brandaris* or *S. haemastoma* alone, since many more than 10,000 snails would be needed, which is already an astounding figure. As mentioned above, some *H. trunculus* snails can produce red-purple dyeings while others blue-purple, whereas *B. brandaris* and *S. haemastoma* only yield red-purple dyeings. Thus, pigments from these latter two species were probably added to a *H. trunculus* bath when needed to produce even redder purples than the *H. trunculus* snails alone can provide. An interesting example that has recently been observed of this possibility is the red-purple fibers in a Late Roman-Period textile whereby these fibers were mostly dyed with *H. trunculus* pigments with an additional contribution from one of the other two snail species (*3*).

Archaeological evidence also exemplifies the preponderance of the usage of *H. trunculus* over that of *B. brandaris* and *S. haemastoma*. In both Aegean and Levantine sites, many more *H. trunculus* shells are usually found than the other two species (*41*, *42*). In addition, all the archaeological purple paint pigments discovered as well as the archaeological and historical textiles that have been chemically analyzed via a full chromatographic analysis have all shown the presence of all three indigoids, with some minor components (*2*, *3*, *6*, *43*). Koren already established that the significant presence of MBI is a chromatic biomarker for the use of the *H. trunculus* species, alone or with some minor additions of one or more of the redder producing snails. Since, as explained above, no significant photo-debromination would have occurred during the anaerobic pre-dyeing stage of the bacterial reductive dissolution of the pigment, any MBI present in a sample, and certainly any IND, would not be as a result of the photo-debromination of the DBI, which is the main colorant in *B. brandaris* and *S. haemastoma*. Hence, all archaeological molluskan purple colorants – pigments and dyes – found to date are a result of the use of *H. trunculus*, alone or with some addition.

Textile Dyeing

The dyeing process commences when the indigoids are properly reduced after all the above-mentioned steps have been followed. The typical color of the naturally reduced solution is greenish due to the combination of the yellowish reduced and dissolved indigoids together with the dispersed undissolved blue indigo particles; hence blue and yellow will produce a greenish coloration.

Dyeing of the textile actually consists of two steps: textile immersion into the vat containing the reduced leuco indigoid dyes followed by air-oxidation of these reduced dyes to their original relatively insoluble state (see also Figure 4).

Immersion of the Textile

Pliny describes the dyeing as such:

> After about nine days the cauldron is strained and a fleece that has been washed clean is dipped for a trial, and the liquid is heated up until fair confidence is achieved. A ruddy color is inferior to a blackish one. The fleece is allowed to soak for five hours and after it has been carded is dipped again, until it soaks up all the juice.

Though Pliny indicates that a total of about 9 days is needed for the dye vat to be ready for the actual dyeing, as a consequence of the reductive dissolution of the indigoids, in all-natural dyeing experiments (*14*) it was shown that the indigoids were in their dissolved leuco state after about 6 days from the start of this whole process, which began with the cracking of the snail's shell as described above.

Air-Oxidation

The last step is the simplest: the textile is removed from the reduced bath and its greenish color immediately begins to turn to its purple color while undergoing air-oxidation to its original insoluble pigment. In any chemical dyeing, the uptake of the dye by a textile is never 100%. In order to exhaust the expensive dye bath as fully as possible, after the textile was dyed and the dissolved reduced leuco-dye re-oxidized in air to its original oxidized state, the textile is then re-inserted into the dye bath in order to obtain a richer and darker color. This is in accordance with the latter part of the afore-mentioned excerpt from Pliny:

> The fleece is allowed to soak for five hours and after it has been carded is dipped again, until it soaks up all the juice.

Talmudic Parallels to Pliny

Pliny the Elder wrote a detailed accounting of the practice of Tyrian Purple or Imperial Purple as practiced in the Roman Period. A much more succinct, but parallel recounting of the method by which *Tekhelet* – the "Biblical Blue-Purple" dye – was produced is noted in a Jewish source, specifically in the Babylonian Talmud (*44*). This woolen dye and another biblical dye, the red-purple *Argaman*, were both produced from sea snails. The passage is dated to the early 4th century CE, just a few centuries after Pliny's work, with parenthetical explanations given by the author in square brackets:

> Abbayei said to Rabbi Samuel son of Rabbi Judah: 'That Tekhelet, how do you dye it?' He said to him: We bring sea snail 'blood' [the red-purple pigment] and substances [*'samanin'* in Aramaic-Hebrew] and put them into a vat (and we heat [literally *'boil'*] the mixture). We then take out a little [of the liquid] into an egg-shell and test [the liquid] with a fleece of

wool. We then throw away that egg-shell and burn the [trial sample of dyed] wool.

The word *'samanin'* as used throughout the Talmud has referred to various substances. These materials have included the following: dyes, dyestuffs, and pigments; drugs, medicines, medicinal ingredients, and wound ointments; and as cleansing and laundering substances. In the latter case, seven substances are itemized for the purpose of removal of a blood stain on a cloth in order to differentiate it from a dyed area – the dye will not be removed. The substances included are alkaline materials, including *neter*, which is synonymous with natron (sodium carbonate).

From this current investigation of Pliny's description, it is then obvious that the Talmud's mention of "substances" (*samanin*) in the context of a dyeing auxiliary for *Tekhelet* and Pliny's salt (*salem*) are equivalent and perhaps even identical. Besides water, all the ingredients, except one, are contained within the snail as implied by both Pliny's and the Talmud's descriptions of the molluskan dyeing practice. These include the extracted purple pigment, the bacteria and their meaty nutrients. The only external reagent needed to produce a successful reduced dye bath was Pliny's *salem* or the Talmud's *samanin*, which in both instances, was an alkaline substance, such as one of the salts mentioned above.

The Origin of Purple Dyeing

There has been much discussion as to where and when did the processing of the molluskan purple pigment begin (*41*, *45*). The more recent trend is to assign the origin of this biotechnological industry to the ancient Minoans and related peoples from the Aegean area or from Italy (*41*) from perhaps as far back as four millennia ago. In the not-so distant past, the previous popular notion was that the Levantine Phoenicians were the ones who developed the methods by which this purple pigment can be utilized. Based on that latter assignation, the famous term "Tyrian Purple" was coined after Tyre, one of the important ancient Phoenician cities where purple was produced.

Those who credit the inhabitants of the Aegean area indicate that there is evidence for "purple dye production" that pre-dates that from the Levantine coast. The claim is that there is "direct evidence" for the production of the purple "dye" from the various broken or crushed *Muricidae* snails that have been found at various sites in the Aegean. The scientific problem with this assertion is that this is not "direct" evidence that can be upheld in a court of scientific law, but rather indirect evidence alluding to the fact that these shells may have been used for the production of the purple pigment.

Various authors have misused the word "dye" to indicate any colorant. It is therefore imperative that those who write about the origin of purple dyeing understand the difference between "pigment" and "dye". While they can be both generically referred to as "colorants", a "pigment" is a substance that is essentially insoluble in water, whereas a "dye" is water-soluble. In order to perform a true dyeing of a textile – and not just to paint its surface – the colorant must be dissolved

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in an aqueous solution so that the individual dye molecules can penetrate into the interior of the textile fibers and then form strong physico-chemical bonds with the fibers. In this way, the dye will be fast, i.e., will be stable and will not wash out of the textile when laundered. When the colorant is a pigment, it cannot be used in dyeing unless it is converted to a soluble form, and then that pigment becomes a dye. For example, in the current study, indigo and its brominated derivatives that constitute the molluskan purple pigment, if they were to be used as true dyes, must be solubilized by reducing them to their soluble reduced leuco-form, as mentioned above, and in that state they function as "dyes" for the dyeing of textiles. On the other hand, if a pigment is left as such in its water-insoluble state without further processing, it cannot be used for the dyeing of textiles – an internal phenomenon – but only in the painting of objects – an external, surface treatment. Examples of the latter are wall paintings or frescoes, painting of vessels, and perhaps even in the painting of textiles that are to be used as burial shrouds, where they will not of course be washed and thus without the possibility that the colorant will wash off the substrate.

Hence, the molluskan purple colorant of this study could have been used as a pigment for the surface coloration of objects, or, if further processed to a soluble form, could have been used as a dye for the dyeing of textiles. In archaeological sites whereby deliberately broken or crushed *Muricidae* sea snails have been found, it is a safe conjecture to state that these snails were probably not destined to be used as sea food, as the broken shells would have gotten attached to the meaty flesh of the snail, which would have made eating them difficult (*46*). However, finding broken shells does not automatically imply that these snails were processed for dyeing of textiles. Broken shells can simply mean that most probably these snails were used to produce the purple pigment. What will then happen to that pigment is a different story: it could be used as a paint pigment, once cleaned from the snail meat to which it adheres, or, if the intention is there, to be further processed and reductively dissolved into a dye form for the dyeing of textiles. Hence, finding broken shells is not "direct evidence" of purple dyeing; it is simply circumstantial evidence that, most probably, a pigment was produced.

The only direct evidence that the relevant sea snails were processed at a certain site for textile dyeing is from physical and chemical evidence of the presence of a purple residue whose source is molluskan. After dyeing of a textile was performed, the remaining dye in the bath eventually becomes oxidized to the solid pigment, which will adhere to the interior walls of the vessel. As mentioned above, the indigoid pigments are so water-insoluble that after more than three millennia visibly noticeable residual staining can still be seen. In a number of Phoenician sites along the Levantine coast of the Eastern Mediterranean, various purple-stained potsherds, fragments from vats, have indeed been found, as can be seen for example from Figures 5 and 9. Chemical analyses of these purple residues have determined that the source of that purple pigment is indeed molluskan. The oldest such purple-stained potsherds date from about the 14th century BCE (*41*). This then constitutes "direct evidence" that at this site, purple dyeing of textiles was practiced. The chemical requirement that a full multi-component chromatographic analysis of that pigment be performed is essential for the determination if "purple dye production" was performed.

In spite of the fact that numerous sites in the Aegean have shown the presence of Muricidae shells, to the knowledge of this author, to date, not a single example of a purple residue has been found at any of these sites. This is most unusual as due to the insolubility of the pigment, if a clay fragment from a true dye vat was found then part of the vat would most certainly show some residual purple stains. The fact that no such example has been found is very telling, and thus there is no chemical evidence, to date, that dyeing of textiles did take place in the Aegean.

The question that then arises is what was the function or purpose of these sea snails whose shells have been found in the Aegean if not for dyeing, and not for food? There is another purpose for these snails other than to produce a dye. They may be used for producing the colorant to be used as a pigment for painting. In fact, there are five archaeological examples of the purple pigment found at various sites in the Aegean where chemical evidence has definitively shown the presence of molluskan purple as a paint pigment, either as already used in the painting or pigment ready to be used (*43*). These samples are the earliest direct chemical evidence and date from the Late Bronze Age (17th century BCE or earlier). They were found in the following Aegean sites: Akrotiri and Raos – both located in the island of Santorini (Thera) – and from Trianda on the island of Rhodes. All of these purple specimens are pigments and not dyes.

The scientific conclusions are that the only bona fide chemical evidence, so far, for the use of purple from the Aegean is as a paint pigment and not as a dye. Hence, researchers who discuss the findings of *Muricidae* shells in the Aegean should not refer to that find as "proof" of "purple dye production". The more correct terminology is to refer to that indirect evidence of finding broken shells as probable indication for "purple pigment production". To date, that is the only find from the Aegean.

The major conclusion of the above discussion is extremely important. Archaeologists have already determined that Aegean sites associated with the Muricidae snails may date from as far back as four millennia and pre-date those sites from the Levantine coast by a few centuries, where textile dyeing has been chemically proven to have occurred. Until chemical evidence points to the contrary – i. e., if a purple-stained potsherd from a vat will be found in the Aegean whereby chemical analyses prove that the source of the purple pigment is molluskan – only the following conclusion can be scientifically drawn: The extraction of the purple pigment from sea snails to be used as a paint pigment probably originated in the Aegean; however, the transformation of that purple pigment to a dye for the dyeing of textiles probably originated later by the Levantine Phoenicians.

Conclusions

Archaeological artifacts that have survived the ravages of thousands of years of hoary history have borne witness to the multiple stages associated with the overall dyeing process using molluskan purple pigments. This critical study has found that the detailed description of these procedures given by Pliny the Elder is relatively complete and correct, which vindicates Pliny's veracity on the subject,

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2,000 years after he documented them. Understanding the complexity of this dyeing process lies in its simplicity: all the steps involved can be explained by invoking the elementary principles of chemistry.

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References

1. Spanier, E.; Karmon, N. In *The Royal Purple and the Biblical Blue: Argaman and Tekhelet. The Study of Chief Rabbi Dr. Isaac Herzog on the Dye Industries in Ancient Israel and Recent Scientific Contributions*; Spanier, E. Ed.; Keter: Jerusalem, Israel, 1987; pp 179−192.
2. Koren, Z. C. *Microchim. Acta* **2008**, *162*, 381–392.
3. Koren, Z. C.; Verhecken-Lammens, C. *e-Preserv. Sci.* **2013**, *10*, 27–34, and references therein.
4. Cooksey, C. J. *Molecules* **2001**, *6*, 736–769.
5. Cooksey, C. *Sci. Prog.* **2013**, *96*, 171–186, and references therein.
6. Koren, Z. C. In *Cultural Heritage and Archaeological Issues in Materials Science*; Sil, J. L. R., Trujeque, J. R., Castro, A. V., Pesqueira, M. E., Eds.; Materials Research Society Symposium Proceedings; Cambridge University Press: New York, 2012; Vol. 1374, pp 29–48, and references therein.
7. Pliny, *Natural History*; Rackham, H., Transl.; The Loeb Classical Library; Harvard University Press: Cambridge, MA, 1940; Vol. 3, Book 9.
8. Vykukal, R. L. Master's Thesis, University of Tennessee, Knoxville, TN, 2011. http://trace.tennessee.edu/utk_gradthes/1034.
9. *Pliny the Elder. The Natural History*. Bostock, J.; Riley, H. T. Transls.; Taylor and Francis: London, 1855. http://www.perseus.tufts.edu/hopper/text?doc=Perseus:text:1999.02.0137.
10. *Pliny the Younger. Letters. XXVII. To Baebius Macer*; The Harvard Classics, 1909–14. http://www.bartleby.com/9/4/1027.html.
11. *Pliny the Younger. Letters. XXVII. To Baebius Macer.* The Project Gutenberg EBook of Letters of Pliny. http://www.gutenberg.org/files/2811/2811-h/2811-h.htm#link2H_4_0028.
12. Pliny the Elder. *Wikipedia*. http://en.wikipedia.org/wiki/Pliny_the_Elder.
13. Natural History (Pliny). *Wikipedia*. http://en.wikipedia.org/wiki/Natural_History_(Pliny)
14. Koren, Z. C. *Dyes Hist. Archaeol.* **2005**, *20*, 136–149, and references therein.
15. Padden, A. N.; Dillon, V. M.; John, P.; Edmonds, J.; Collins, M. D.; Alvarez, N. *Nature (London)* **1998**, *396*, 225.
16. Padden, A. N.; Dillon, V. M.; Edmonds, J.; Collins, M. D.; Alvarez, N.; John, P. *Int. J. Syst. Bacteriol.* **1999**, *49*, 1025–1031.
17. John, P.; Arghyros, S.; Nicholson, S. *Dyes Hist. Archaeol.* **2008**, *21*, 45–50.
18. Nicholson, S. K.; John, P. *Appl. Microbiol. Biotechnol.* **2005**, *68*, 117–123.

19. Compton, R. G.; Perkin, S. J.; Gamblin, D. P.; Davis, J.; Marken, F.; Padden, A. N.; John, P. *New J. Chem.* **2000**, *24*, 179–181.
20. Park, S.; Ryu, J.-Y.; Seo, J.; Hur, H.-G. *J. Korean Soc. Appl. Biol. Chem.* **2012**, *55*, 83–88.
21. Koren, Z. C. *Isr. J. Chem.* **1995**, *35*, 117–124.
22. Haigh, J. *The Dier's Assistant in the Art of Dying Wool and Woollen Goods. Extracted from the Philosophical and Chymical Works of Those Most Eminent Authors Ferguson, Dufay, Hellot, Geoffery, Colbert; and That Reputable French Dier Mons. de Julienne. Translated from the French. With Additions and Practical Experiments*. James Humphrey's: Philadelphia, PA, 1810; p 31.
23. Haigh, J. *The Dier's Assistant in the Art of Dying Wool and Woollen Goods. Extracted from the Philosophical and Chymical Works of Those Most Eminent Authors Ferguson, Dufay, Hellot, Geoffery, Colbert; and That Reputable French Dier Mons. de Julienne. Translated from the French. With Additions and Practical Experiments*. James Humphrey's: Philadelphia, PA, 1810; pp 54, 55.
24. Baig, G. A. *AUTEX Res. J.* **2010**, *10*, 21–25.
25. McConnell, T. H. *The Nature of Disease: Pathology for the Health Professions*; Lippincott Williams & Wilkins: Baltimore, MD, 2007; p 490.
26. Alcamo, I. E.; Krumhardt, B. *Barron's Anatomy and Physiology the Easy Way*, 2nd ed.; Barron's Educational Series: Hauppauge, NY, 2004; p 442.
27. Sandberg, G. *Indigo Textiles: Technique and History*; A & C Black: London, 1989; pp 171, 172.
28. Dillard, C. R.; Goldberg, D. E. *Chemistry Reactions, Structure, and Properties*, 2nd ed.; Macmillan: New York, 1978; p 203.
29. Haynes, W. M., Ed.; *CRC Handbook of Chemistry and Physics*, 93rd ed. (Internet Version 2013); CRC Press/Taylor and Francis: Boca Raton, FL, 2013; pp 5−196.
30. Kotz, J. C.; Treichel, P., Jr. *Chemistry & Chemical Reactivity*, 4th ed.; Saunders College Publishing: Fort Worth, TX, 1999; p A−28.
31. Weast, R. C., Ed.; *CRC Handbook of Chemistry and Physics*, 66th ed.; CRC Press: Boca Raton, FL, 1985−1986; pp B-82, B-222.
32. Calcium carbonate. *Wikipedia*. http://en.wikipedia.org/wiki/Calcium_carbonate.
33. Geyssant, J. In *Calcium Carbonate: From the Cretaceous Period into the 21st Century*; Tegethoff, F. W., Rohleder, J., Kroker, E., Eds.; Birkhäuser Verlag: Basel, Switzerland, 2001; pp 1–52.
34. Naydowski, C. In *Calcium Carbonate: From the Cretaceous Period into the 21st Century*; Tegethoff, F. W., Rohleder, J., Kroker, E., Eds.; Birkhäuser Verlag: Basel, Switzerland, 2001; pp 197–237.
35. Yaalon, D. H. *Plant Soil* **1957**, *8*, 275–288.
36. Koren, Z. C. *Dyes Hist. Archaeol.* **1993**, *11*, 25–33.
37. Livy. Book 21, Chs. 30-38, Secs. 30.1, 37.2, 37.3. http://www.johndclare.net/AncientHistory/Hannibal_Sources3.html.
38. Marin, F.; Luquet, G. *Mater. Sci. Eng. C* **2005**, *25*, 105–111.

39. Marion, G. M.; Millero, F. J.; Camões, M. F.; Spitzer, P.; Feistel, R.; Chen, C.-T. A. *Mar. Chem.* **2011**, *126*, 89–96.
40. Garrison, T. *Oceanography: An Invitation to Marine Science*, 7th ed.; Brooks/Cole, Cengage Learning: Belmont, CA, 2010: pp 195–196.
41. Reese, D. S. *Mediterranean Archaeol. Archaeom.* **2010**, *10*, 113–141.
42. Reese, D. S. In *Kommos IV. The Greek Sanctuary, Part 1*; Shaw, J. W., Shaw, M. C., Eds.; Princeton University: Princeton, NJ, 2000; pp 643−645.
43. Karapanagiotis, I.; Mantzouris, D.; Cooksey, C.; Mubarak, M. S.; Tsiamyrtzis, P. *Microchem. J.* **2013**, *110*, 70–80.
44. Babylonian Talmud, Tractate *Menahot*, p 42b.
45. Stieglitz, R. R. *Biblical Archaeol.* **1994**, *57*, 46–54.
46. Çakırlar, C.; Becks, R. *Studia Troica* **2009**, *18*, 87–103.