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HOW TO MOUNT AN INEXPENSIVE SIEVING LAB

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ABSTRACT

We present a new sieving technique designed to recover microfossils from mudstones, clays and poorly consolidated sediments. This new technique is designed to be inexpensive, use the minimal amounts of chemicals and recover well preserved microfossils. The inexpensive nature of this methodology makes it suitable for reconnaissance studies, where finances may be limited. Not using large amounts of chemicals helps to protect the fossils and the environment. Investigations on the Jurassic Lourinhã Formation, Portugal yielded a diverse microfossil assemblage including; archosaur teeth, lizard jaws, amphibian jaws, fish remains, ostracods and charcoal. Such a diverse fossil recovery shows the technique is suitable for painstaking palaeoecological studies as well as reconnaissance work.

RESUMO [in Portuguese]

É apresentada uma nova técnica de crivagens para recolha de microfósseis de pelitos, argilas e sedimentos mal consolidados. Esta nova técnica foi concebida para ser o mais económica possível, usando uma reduzida quantidade de químicos e permitir recolher microfósseis bem preservados. A natureza económica desta técnica permite efectuar estudos de reconhecimento. O uso reduzido de químicos protege os fósseis e o ambiente. As investigações feitas na Formação da Lourinhã (Jurássico, Portugal) compreendem uma rica diversidade de microfósseis incluindo: dentes de arcossauro, mandíbulas de lagartos e anfíbios, restos de peixes, ostracodos e madeira fossilizada. A rica diversidade de fósseis recolhida demonstra que a técnica é indicada para estudos paleoecológicos bem como para trabalho de reconhecimento.

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INTRODUCTION

Sieving methods for microvertebrate recovery have rarely (Mateus et al. 1997) been applied to the Lourinhã Formation. The Lourinhã Formation is comprised of fossiliferous fluviodeltaic deposits that outcrop extensively in the Lusitanian Basin, Western Portugal. This formation is mainly composed of intercalated sandstone channels with extensive alluvial mudstone layers (Hill 1989). Within the extensive mudstone layers most fossils including many large vertebrates (Antunes and Mateus, 2003) and microfossils are found (e.g. Ramalho 1967). Microvertebrate fossil faunas have been collected for many years in the Lourinhã Formation, mainly by surface collecting. However, in 2008 the Museum of Lourinhã started a systematic sieving campaign to better understand the overall fauna of the Lourinhã Formation.

Some of the first microvertebrate finds in Portugal were discovered in the Guimarota mine in 1960 by palaeontologists from the Freilicht Universitat, Berlin (Krebs, 2000). The first account of sieving methods being used in Portugal were published by Kühne (1968), using a constant flow of water and sieves incorportated on a metal barrel. Sieving was applied at the Paimogo theropod embryo nest site (Mateus et al. 1997) from 1994 to 1996, in the search for embryo bones and eggshells (Mateus, 1998) and was also occasionally applied to the Porto das Barcas fossil site, but without much success.

Precise geographical information of sieving sites in the Lourinhã Formation was acquired using GPS coordinates, stratigraphic information and a measured section was acquired by plotting the site photograph with detailed geologic annotation. Samples were taken using a pick axe at 30 - 40cm stratigraphic intervals and within 1 - 2m horizontal spacing. This systematic sieving campaign, applied to the Lourinhã Formation for the very first time, is being used to investigate and assess the composition and diversity of the microfossil fauna.

The first use of sieving in the search of microvertebrate remains was performed *ca*. 1847 by Plieninger, in Germany (McKenna et al. 1994). Early methods of sieving were also used by Moore in England (1867) and later by Wortman and Brown in the United States (1891). Sieving became well implemented in the palaeontological community after Hibbard (1949) reported using the technique to collect Cenozoic mammal fossils from an unconsolidated sandstone matrix (McKenna et al., 1994). Hibbard introduced the use of screen boxes (wooden boxes with a brass mesh). However, this method requires having water near the work site, is laborious, and requires a large staff (Ward 1984). McKenna (1962; 1965) provided further insight into the screen box technique and proposed a standard model using manufactured rectangular wooden boxes and a regular size steel mesh. Both McKenna's and Hibbard's techniques are field-oriented and require the presence of nearby water. McKenna simply optimized Hibbard's technique by processing a larger quantity of matrix using more screen boxes (almost 300, compared to Hibbard's dozen), and adapting the method to the constraints of particular sites. Grady (1979) described a new method using mosquito nets instead of the classical material of screen boxes with brass mesh, providing a more field-oriented method with easily transported equipment (Ward, 1984).

In contrast, the method reported in this paper is similar to other laboratory-oriented techniques. Described in further detail by Kühne (1971) and Krebs (2000), the "Henkel technique" (Henkel, 1966) is a laboratory-oriented technique that was used for more than a decade during the time that microvertebrates were being collected from the Guimarota Mine. This technique is a static sieve method making use of a jet of water that passes through a barrel with a 500µm -mesh on the side. Freudenthal (1976) developed a table technique. This "table" stood 1m high and used a 500µm mesh as a replacement for the table top. Solid side bars were attached in order to avoid sediment loss. A jet of water was used to wash the sediment through the table top.

The methods above described provide excellent guidance for new field researchers, but due to peculiarities of each site/investigation, modifications to the methods may be required. Aspects such as the location of the fossiliferous horizon (i.e. remoteness), budget and laboratory conditions (i.e. pre-existing infrastructures) can hinder recovery of specimens in an identifiable condition. The methodology here described does not aim to process vast amounts of sediment, as others can. Instead, it does allow individual horizons to be processed without the potential of mixing sedimentary beds. When a sedimentary bed is of limited vertical extent the field-orientated techniques require extensive excavation of the target horizon, losing possible valuable horizons, or mixing multiple sedimentary layers. This can hinder the investigation of fine

scale ecological, climatological and evolutionary patterns.

MATERIALS

The list below is the equipment used for this technique during the 2008 Museu da Lourinhã field season with approximate prices (see fig. 1):

- Glass bottles (donated free by local cafés)
- Fine paint brushes (diameters 0.25 to 0.5cm) 1,5€
- Sieves of different mesh sizes (15000µm, 750µm, 500µm. Sizes estimated using a grain size comparator chart) - four of each 22,5€
- Plastic bowls (four sets of circular: 40cm, 30cm, and squared: 30x30cm. Different coloured sets provides an easy way to avoid mixing up samples during processing) 19€
- Plastic Trays (20, for drying, 20cm in diameter) 9€
- Latex gloves (one pair per worker per day, 200 pairs) 2,5€
- Hard - water softeners like polycarboxylates (e.g. Calgon®) (10 - 30g/day) 4€
- Packet of Self - adhesive labels 1,5€
- Funnels (two) 1,5€
- Metal Ashtrays (Two, 10x10cm) 1,5€
- One garden trowel 1,5€
- **TOTAL** **63€**

In addition, supplementary laboratory supplies and equipment are required:

- Hand lens (~10€), Respirators (~30€), Washbasin (preferably with shower), Lab coat(s), hydrogen peroxide (1l, 5%v/v), Optical microscope (1000 - 4000€, Magnifications range 0,63 to 8,65X).

Figure 1. Materials used for sieving: A-- transparent bowls used to dissolve the boulders collected in the field; B- 15000µm sieve; C-garden shovel used to move sediment between bowls; D- 750µm sieve; E- various small-sized bowls (30x30cm); F- large-sized bowl (30cm diameter); G-trays to dry sediment; H- painted ashtray for picking, and various brushes; I- labeling material; J- small transparent boxes used to store sorted specimens and glass juice bottle used to store unpicked sediment; K- binocular microscope.

METHODOLOGY

1. Approximately 45 rock samples were collected, each sample weighing approximately 5 kg. We took into consideration precise geographical and stratigraphic location, broad objectives for scientific outputs, and sediment trauma. The rock samples were stored in sealable polythene bags (Ziplock®) to avoid any contamination and were labeled using a water resistant black marker pen.
2. To start the sieving process, a 30cm diameter plastic bowl (Fig. 2A) was filled with hot water, at a temperature below boiling (60 to 70ºC) in order to avoid weaken the structural integrity of the plastic.
3. 3.Next, 5 to 20 ml of hydrogen peroxide was added to the hot water, different quantities can be added depending on how compact the sediment is. The use of hydrogen peroxide has been shown to be an effective way of liberating clay minerals from microvertebrate specimens without the damaging effects of other commonly used chemicals, such as acids (Wilborn, 2009).
4. A 15000µm sieve was placed into the bowl of hot water and as much sample as possible was put into the now submerged sieve, typically around 500 grams (Fig. 2B).
5. The sediment disaggregated without disturbance (Fig. 2C). This process normally took about 30 minutes.
6. If the sediment was still compact after 30 minutes. The water was changed for fresh hot water, except for a 1cm layer above any disaggregated sample (to avoid any loss), and 5 - 20 ml of hydrogen peroxide was added.
7. Once there was only a little sediment (and perhaps some fossils) left in the 15000µm sieve, the sediment in the sieve was emptied onto a tray. Each tray used was labeled with the appropriate sample number and left to dry in the sun. Spotlights or a radiator within the laboratory can achieve the same result by increasing the temperature.
8. The 40cm diameter plastic bowl was filled with cold water. A small garden trowel was used to transfer the sediment to the 750µm sieve. Using a trowel or any similar tool instead of hands is an effective way to transport sediment without loss. The sieve was filled to the top as it proved quicker to sieve larger portions of the sample than smaller ones (Fig. D, E, F). Agitation was performed with the sediment continually immersed in water as the sieve was shaken by hand in circular movements. As the water became cloudy it was necessary to replace it, taking care not to lose any of the sieved material. When refilling the bowl caution should be taken in directing the flow of water against the side of the bowl, thus causing minimal agitation to the sieved material. Once the sample within the sieve was considered clean (no visible clay coloured streak appeared in the water when shaking) it was transferred to a labeled plastic tray. Any objects trapped within the sieve mesh were gently freed by gently tapping the sieve (Fig. 2 G, H).
9. Once the sample was sieved through the 750µm sieve, the remaining proportion of the sample (the <750µm portion of the sample) is left in the bowl and the fraction from the sieve was transferred to a plastic tray. The tray was labeled and left to dry (Fig. 2I).
10. The <750µm portion was then sieved again with a 500µm sieve. After this step, there remained a portion of the sample with a grain size under 500µm.
11. The <500µm portion was washed using a shower nozzle with low pressure. The operator used his hand to create turbulence within the plastic bowl to carefully put the sediment into suspension, helping to liberate the clay fraction. The <500µm fraction was allowed to dry.
12. Once all the sieved samples were dried they were transferred to appropriate containers for study under the microscope. The containers used for this field season were glass juice bottles that were washed, cleaned with water, and dried prior to this procedure. The containers were labeled using adhesive labels for outside the bottle and a small slip of paper within the bottle (Fig. 2K).
13. In the case a sample needs to be left part-way through processing, it is advised that the water used during sieving be drained and replaced with fresh cold water. This way the clay does not dry overnight which can cause clumping of the sediment.

Figure 2. Some aspects of the sieving methodology: A-- Lab during the sieving season (note the different colored bowls), B-- the clay boulders are left to disaggregation in the 15000µm sieves, C-- a dose of hydrogen peroxide can be added if the boulders are hard to dissolve, D-- with a garden shovel the sediment is transferred to the 750µm sieve, E-- the bowl to which the sieved sediment will go to can be filled with cold water, F-- sediment passes through the sieve with circular movements in the water, G-- in order to minimize the amount of time sieving, small parcels of sediment should be done at a time, H-- sediment passes through the 500µm sieve, I-- the sediment is left to dry at atmospheric conditions, J-- if the weather does not permit the sieved sediment can be left to dry under strong light, K-- store the dried sediment in juice glass bottles, L-- by the end of the day clean the pipes using deflocculating agents and hot water.

SAFETY

The major risk associated with this technique is when handling the hydrogen peroxide. Gloves, respirator, lab coat and boots should be used by the operator. The process is best done in pairs to allow one person to poor the hydrogen peroxide and another to ensure there is no spillage.

To avoid sample cross contamination sieves should be cleaned after they are used. Washing the sieve in water and using a stiff brush is typically enough to remove most particles. Whilst a toothpick can be used to free the most stubborn quartz grains from a sieve mesh.

Because large amounts of sediment were dumped into the laboratory sink, hard - water softeners (e.g. Calgon®) and hot water were poured down the drain at the end of each working day. To further prevent blockages from occurring, the trap below the sink was emptied and manually cleaned regularly (Fig. 2L). An alternative to dumping residue down the drain is to let it settle in the basin overnight, and decant the water off the top down the drain. Let the residue dry and throw it away.

To handle hydrogen peroxide should be handled with acid gloves, acid-proof glasses, and an acid-resistant rubber coat.

DISCUSSION

Contrary to static sieving methods discussed by Ward (1981), this technique uses considerably less water, thus, it is inexpensive and environmentally friendly. Our method uses only 10 - 15l per sieve. The technique described here allows samples to be collected during a field campaign and processed at a later date. Making it suitable for seasonal regions where short, intense fieldwork is only possible during warmer/dryer parts of the year. It is also an excellent technique for a reconnaissance sampling by investigating the potential of a new site without excessive time or money being invested.

Nevertheless, this technique could be easily adapted to remote areas. By using jerrycans for water storing water, and by using the plastic bowls and kitchen sieves (widely available in major cities), the technique here described could be employed with little modification. Only the lack of running water would make it difficult to process sediment <500µm, but the rest of the sediment could be greatly reduced in weight for transport. In this experiment, approximately 200 kg was processed by four volunteers sieving for two weeks working 4 to 5 hours a day. In our case, three series of sieves were used simultaneously, which allowed processing two, and in some cases three 5-kg samples a day, depending on the nature of the sediment.

Although no number of sieves and people is specified in McKenna et al. (1994), the technique here described processes approximately 45 kg per day, compared to 1800kg per day of other techniques. The severity of this limitation is not clear, because no specific information is available in the literature for comparisons. Nevertheless, the diversity of fossils recovered in fossiliferous layers was remarkable: each 5-kg sample produced fish remains (e.g. scales, teeth); amphibian and lizard jaws; archosaurian teeth and ostracods. As a negative control, oxidized paleosols, known to be poorly fossiliferous, barely produced any fossils when using the same technique. Although not quantified under controlled experiments, the amount of time that should be spent on sieving is a tradeoff between concentrating and separating grains of appropriate size and preservation state of the fossils (since the abrasion during sieving could potentially damage the fossils).

It is difficult to assess the amount of information from different techniques based on the yield, compared to the time required and amount of money invested. To make a fair assessment of information gained from each methodology, when compared to its time and financial investment, would require a comparison of techniques based on the same sedimentary horizon. In turn this would also require different lithologies and horizons to be processed to assess the most efficient technique for different geological settings. However, there is still the risk of confounding the intrinsic productivity of the method instead of the method efficiency, yield amount being only one factor of method efficiency. A qualitative and quantitative analysis of the fossil damage or number of rare taxa recovered, under different sieving methods, could serve as proxies for the method efficiency. These comparative studies will be attempted in the future seeking to determine which sieving technique should be used under what situation, but are beyond the scope of this paper.

For each 5kg sample, the 750µm and 500µm sieve fractions recovered well-preserved fossils, with no fracturing or damage. Whereas techniques described by Hibbard (1949), McKenna (1962, 1965) and McKenna et al. (1994) imply that the loss of specimens smaller than the mesh size is unavoidable (since sediment below the smallest sieve is dumped), the technique described in this paper recovers specimens of almost all sizes, even enabling the collection of small ostracods and carophytes (100 - 200µm), which are useful for paleoecological reconstructions. Finally, this methodology does not require an extra step of concentration of the residue by use of acids or heavy liquid flotation (Gibson and Walker 1967), which is known to cause damage potentially to both the desired fossils and the environment (Cifelli et al. 1996).

In essence the "Henkel technique" and the method here described share the "static sieve" idea, but the technique described by Henkel (1966), although hard to compare, seems to be more aggressive to the fossils (see figs. 2 and 3 in Rauhut, 2001, showing many tooth apex broken) and it does not retain below mesh sized matrix.

The method described in this paper is designed to be an inexpensive technique, to recover the maximum amount of microfossils with a

minimal amount of damage and cost. This method is also designed to use minimal amounts of chemicals. This helps to prevent chemical damage to specimens, which can obscure or destroy textures on fossils (Cifelli et al. 1996).

A major step to improve this technique could be to adapt it to process larger amounts of material. This could be accomplished by using larger sieves, which would not influence greatly the final budget. Following McKenna's technique, although inexpensive, it does not adapt to laboratory conditions and requires the construction of sieves.

CONCLUSIONS

The main advantage of this method is the low cost, therefore: 1) the technique here presented seems to be an excellent exploratory or small project procedure. 2) all the materials needed are easily accessible in any regular store. 3) it uses the absolute minimal amounts of chemicals – preventing damage to fossils and improving safety for volunteers, 4) the material recovered from the Lourinhã Formation is in an excellent state of preservation.

The main drawback is the small amount of sediment that can be processed (45kg/day, 4 volunteers working 4-5 hours) making this technique more suitable to smaller studies or reconnaissance investigations.

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