

## Moisture Inhibits the Decomposition Process of Tissue Buried in Sea Sand: A Forensic Case Related Study

Franklin R.W. van de Goot<sup>1,2\*</sup>, Mark P.V. Begieneman<sup>1,2,4</sup>, Mike W.J. Groen<sup>1,3</sup>, Reza R.R. Gerretsen<sup>1,3</sup>, Maud A.J.J. van Erp<sup>2</sup> and Hans W.M. Niessen<sup>2</sup>

<sup>1</sup>Centre for Forensic Pathology B.V. 3741 GK Baarn, the Netherlands

<sup>2</sup>CaR-VU, Department of Pathology and Cardiac Surgery VU Medical Centre, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands

<sup>3</sup>Barge's Anthropologica, Department of Anatomy, Leiden University Medical Centre, Eindhovenweg 20, 2333 ZC Leiden, the Netherlands

<sup>4</sup>Netherlands Forensic Institute (NFI) Laan van Ypenburg 6, 2490 GB the Hague, the Netherlands

### Abstract

Many aspects influence the decomposition process of a body and, as such, are important in forensic science for estimation of the post mortem interval. In a recent forensic case, a missing man was found buried in sea sand. The post mortem interval estimation as obtained at autopsy was quite different to the actual period this man was missing. In the present study, we have set up an artificial decomposition model to study the effect of sea soil and moisture, relevant to this particular case, on the decomposition mode.

Pig (*Sus domesticus*) legs were buried in 50 litres of sea sand and control sand (woodland sand) respectively, within containers for 1, 2 and 3 months. The sand was evaluated using routine pedological analysis. The legs were analysed using AZAN staining and microscopically scored for their decomposition grade. In the second part of the study, the effect of moisture hereon was analysed.

Pedological analysis did not show significant differences in composition between the sea- and woodland sand. Although an increase in decomposition grade was found in both soils over time, no differences in decomposition grade were found. In the second part of the study, however, we found a significant decrease in decomposition score in legs buried in wet, soaked sea sand compared to those buried in dry sea sand. Soaked means a small layer of water was seen on the container's surface.

We have successfully developed an *in vitro* decomposition model in order to address taphonomic questions related to a forensic case and have found that moisture inhibited the process of decomposition in sea sand.

**Keywords:** Taphonomy; Decomposition; Moisture; Histology; PMI

### Introduction

Taphonomy is the science of the laws of embedding [1] combining geological, biological and historical approaches into one methodology [2-15]. Forensic taphonomy integrated the concept of taphonomy within the field of forensic science. This is applied in the study of human decomposition, the determination of the Post-Mortem Interval (=PMI) or Post-Burial Interval (= PBI), the analysis of the cause and manner of death, and the distinction between products of human behaviour from those created by natural factors such as, for instance, animal activity and the identification of human remains sites [16-18].

Decomposition on a macroscopic level is well documented from ancient literature (the Gilgamesh Epic quotes maggots in a human body infestation in a warm environment [19]) to modern literature. In particular, the formation of livor mortis, rigor mortis and temperature are well-documented [20-23]. Nevertheless, these aspects are signs of early decomposition. The ongoing stages, in particular, are less described.

It is known that the longer the post mortem time lapse is, the more difficult it is for adequate histopathological conclusions sites [24]. Most of the articles dealing with post mortem histopathology pinpoint organ pathology and the level of certainty one still has in using histological techniques. Time related degeneration of normal histological structures such as muscle or nerves that has been systematically documented is very hard to find in literature at present.

The dermis consisting of epidermis with several layers of cells, divided by a basal membrane from the dermis, is a collagen rich

structure containing vessels, hairs and glands, subcutaneous fatty tissue and the deeper muscular tissue, along with the superficial and deeper localised arteries and nerves [25]. It is likely that the decomposition of the epidermis is influenced more by the surrounding soil than, for instance, the deep muscles. On the other hand, the case of a complete body bacterial flora from the inside can give rise to other mechanisms of decomposition.

In this study, we investigated the degeneration of several clearly recognisable histological structures over time on a quantitative scale.

Recently we encountered the importance of the above mentioned taphonomic variables in a forensic case of a missing man that was buried in sea sand (for more details, see the Materials and Methods section) in the early spring of 2008 in the western part of the Netherlands. In this particular case, the initial Post Mortal Interval (=PMI) estimation did not coincide with the period the man was missing. This raised the

**\*Corresponding author:** F.R.W. van de Goot, Centre for Forensic Pathology B.V. 3741 GK Baarn, the Netherlands, Tel: +31 35 5430332; Fax: +31 35 5430019; E-mail: [forensischpatholoog@live.nl](mailto:forensischpatholoog@live.nl)

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question of whether this PMI estimation was, therefore, incorrect. To the best of our knowledge however, research related to the effect of sea sand and/or moisture on the decomposition state of a human body as such is lacking. Therefore, we performed a taphonomy study in an *in vitro* decomposition model to analyse whether sea sand preserves organic material better than woodland soil and whether moisture has a significant impact on this process.

## Materials and Methods

### Case report

Remains of an unknown adult male were found in the western part of the Netherlands in the early spring of 2008 during construction work for an infrastructural project. These remains were accidentally removed from the burial pit by a large excavator and transported over a distance of circa 700 metres to a secondary location. The original burial pit with an average depth of approximately 140 cm had been dug in sand of medium texture (0.5-0.25 mm) and marine origin. In the Netherlands, sea sand is often used to improve the ground texture needed for the foundation of roads or other structures. The discovered body was clothed in a normal fashion according to the weather at the time (i.e. 2 thin layers of clothing). The body was severely damaged by the movement of the draglines. Although there were clear signs of decomposition, the hair of the victim was firmly attached to the head, the epidermis was still present and almost no green coloration was seen. Almost no gaseous formation was noticed and fluid blood was still available. Due to the physical state of the remains, the time between death and discovery, the so-called Post Mortem Interval (= PMI), was estimated at approximately one to two weeks (Figures 1a and 1b). Green discoloration of the body was limited directly after excavation. The green discoloration as noticeable on figure 1a, however, rapidly progressed in this way during the time subsequent to excavation. The soft tissues (Figure 1b) had a more or less normal aspect at autopsy. Overall, this was inconsistent with the fact that after his positive identification this man appeared to be missing for approximately three months. If the initial PMI estimation was correct, an explanation for the approximately two-and-half month episode for which he was reported missing had to be found. If the initial estimated PMI was incorrect, however, there should be an explanation for which factors contributed to this exceptionally good preservation of the remains. In order to test this last hypothesis, the present study was performed to analyse whether sea sand preserves organic material better than woodland sand. In addition, the impact of the level of moisture was examined since the late autumn and winter periods (i.e. the period during which the man was missing) in the Netherlands are notorious for their prolonged periods of heavy rainfall.

### Experimental design

Since there are ethical restrictions within the Netherlands for the use of human tissues in taphonomic research, the experiment was performed on pig tissue (*Sus domesticus*). In previous taphonomic studies, it has been shown that pig tissue decomposes more or less identically to human tissue, although this has not been studied in sea sand before [26-37].

In total, 180 pig legs were used for this experiment, a total organic mass of approximately 50 kg. All legs were obtained from an abattoir while still fresh and were cleaned with hot water according to standard slaughter protocols. As a result, no pigs were harmed for the purpose of the study. These legs did not differ significantly with respect to weight and mass (not shown because all samples were taken from the same

place of the legs, approximately 1cm below the skin at the backside of the leg. We assumed that slight differences in weight and size will not give significant alterations).

The experiments took place at the same time of the year - one year after the victim was reported missing. The location of the burial site of the victim was 75 km from the research facility and provided a more or less similar temperature, humidity degree and sea level according to the Dutch meteorological centre who compared tables of precipitation and temperature where established. Although both periods were not identical, the authors accepted the differences as more or less comparable. During a relatively cold 14 day period in January 2009, the containers were loosely covered with industrial plastic and stored in a more protected area. The distance of only 75 km away from the North Sea was accepted climatologically as being similar.

Plastic containers (40 × 35 × 35 cm) were used to store the legs in both experiments. Pig legs with skin attached were buried in two different sands: one consisted of sea sand, identical to the sand in which the remains of the adult man were found (Figure 1c) and the other control sand was woodland sand, sampled in a nearby area (Figure 1d). The choice for woodland sand as control sand was because much of the knowledge regarding the speed of decomposition of buried human bodies within the Netherlands is derived from information from illegal burials in woodland areas. Woodland soil is common yellow soil found in many places in the deeper earth layers in the Netherlands usually after approximately 1 metre of organic soil yellow sand is found, composing of particles transported by the rivers from central Europe and by the last ice age from the north of Europe. Both sands were sampled from the a) (i.e. surface soil), b) (i.e. subsoil) and c) (i.e. parent material) horizons of the soil profile. 2 m<sup>3</sup> of each soil type was used.

Subsequently, two separate experiments were performed. The first experiment (80 legs) was set up to examine the difference in speed of tissue degradation between the wet sea soil and wet woodland soil, therefore independent of the degree of moisture and at identical



Figure 1: Macroscopic pictures of the victim and the *in vitro* experiments.

a) the body of the victim, b) the head of the victim with preserved soft tissue, c) sea sand, d) woodland sand e) sampling of fresh material f) containers outside in open air.

(surrounding) temperatures. The second experiment (90 pig legs) was performed to examine the putative difference in degradation speed of the pig legs between dry and wet sea soil but, once again, both were at identical temperatures. The 10 remaining legs were used as negative controls (Table 1).

During the first experiment, all containers were placed in open air, generating wet sea sand and woodland sand, although the bottom of the container was perforated in order to drain rainwater. First of all, all containers were filled with 10cm of woodland sand or sea sand and then a pig leg was placed on, positioned in such a way that they did not touch the plastic walls of the container. The containers were then covered with 25 cm of soil that was firmly pressed. Ten containers contained pig legs without sand; ten legs were sampled immediately (Figure 1e). Finally, all containers were placed outside, in the open air (Figure 1f) to simulate the conditions of the crime scene. After 1, 2 and 3 months ten legs of both sand types and controls (3 legs after 1 month, 3 legs after 2 months and 4 legs after 3 months) were excavated and sampled (Table 1).

During the *second* experiment, all 90 (2 × 45) containers were filled with sea sand up to 10 cm. The legs were then placed on top of the soil once again positioned in such a way that they did not touch the plastic walls of the container. The containers were then covered with 25 cm of sand that was firmly pressed. In this experiment however, half of the containers were placed in open air, generating wet sea sand, whereas the other half of the containers were loosely covered with plastic, generating sea sand with less moisture than the uncovered containers. Samples were taken after a period of 1, 2 and 3 months; 15 samples each time (Table 1). Due to an irrigation canal in the bottom of the containers, most of the rain water could easily pour away. Nevertheless,

a small layer of water was observed during rainfall, making the sand Saturated. Saturated means a thin layer of water was permanently seen on top of the soil.

### Sampling method

All samples were taken from the topside of the leg and contained both skin and muscle (Figure 1e). After sampling, all samples were fixed in 4% formalin and histologically processed (i.e. dehydrated and embedded in paraffin). After this, six-micrometre thick slides were processed for each sample and stained. For this routine, AZAN staining was performed to visualise connective tissue that was used for the microscopic scoring analysis (see below).

### Microscopic scoring analysis: decomposition score

All slides were analysed and scored microscopically. For this, five different tissue structures were investigated separately, namely: dermis, fat tissue, muscle, blood vessels and nerves. To quantify the state of decomposition a scoring system of 0 to 3 was used. A typical example of the microscopic scoring analysis of the muscle, stage 0-3, has been depicted in figure 2. As such, the score 0 was related to fresh tissue samples (no decomposition), the score 3 was related to an unburied sample after 1, 2 and 3 months (maximal decomposition) dependent on when exactly each particular sample was excavated. It has to be noted that the maximum score of 3 had already been achieved within the unburied control samples after just one month. All scores are explained in more detail in table 2.

### Statistical analysis

The data derived from both experiments were analysed using

a) Type of samples used in the first experiment

Buried / unburied	Control	Buried	Buried	Buried	Unburied	Unburied	Unburied
Time (months)	0	1	2	3	1	2	3
N =	10	20	20	20	3	3	4

b) Type of samples used in the second experiment

Sand type	Dry	Wet	Dry	Wet	Dry	Wet
Time (months)	1	1	2	2	3	3
N =	15	15	15	15	15	15

Table 1: Explanation of the samples of the experiment.

	Score 0 (fresh samples)	Score 1	Score 2	Score 3 (unburied samples)
<b>Dermis</b>	Dermis appeared in finely orientated fibres, the edges were very sharp and there were no lyses of collagen or adnexal structures.	Dermis appeared in finely orientated fibres, and lyses of collagen and adnexal structures occurred occasionally.	Dermis showed clear lyses of collagen and adnexal structures but was still recognisable as dermis.	Dermis was only appearing in massive coagulating lyses of collagen and adnexal structures.
<b>Fatty Tissue</b>	Fat tissue was sharply lined with smooth edges and clear nuclei.	Fat tissue was occasionally not sharply lined or the chromatin pattern of the nucleus was obviously degenerated but without damage to the outer structure of the nucleus.	Fat tissue was clearly showing signs of lyses, but the actual structure of the fatty tissue was easily recognisable.	Fat tissue was almost completely degenerated, appearing only as ghostly contours.
<b>Muscle</b>	Muscle was sharply edged; there was no sign of fragmentation and no degeneration of the interstitial fibres.	Muscle was occasionally less sharply edged and there were minor signs of fragmentation.	Muscle showed clear fragmentation but not all fibres were damaged and/or the structure of the muscle was still recognisable.	Muscle showed massive lyses and as such the muscle structure was now only recognisable by its colour, not by its histological appearance.
<b>Blood vessels</b>	Blood vessels were clearly consisting of several layers of muscle and collagen and the endothelium was undamaged.	Blood vessels were clearly consisting of several layers of muscle and collagen, however with minor signs of lyses and damage.	Blood vessel showed clear lyses, but the actual structure was still recognisable.	Blood vessels appear only as contours. No histological structures were recognisable.
<b>Nerves</b>	Nerves were sharply edged, there were no signs of lyses and the axon structure was undamaged.	Nerves were occasionally less sharply edged, there were minor signs of lyses and the axon structure showed minor deterioration.	Nerves were still easily recognisable but the internal structure showed clear signs of lyses.	Nerves appeared in ghostly contours and no separate capsule or axon structure was identified.

Table 2: Histological aspects of the scoring system.

conventional univariate analysis with proprietary statistical package SPSS, using the Wilcoxon ranking test.

### Meteorological data

To extrapolate the original burial findings of the victim to the decomposition *in vitro* model, meteorological data concerning the temperature and rainfall were obtained from the Royal Netherlands Meteorological Institute (KNMI) in De Bilt. Precipitation amounts in time are represented in figure 3.

It was noticed that a significant amount of rainfall did occur during the period in which the man went missing, namely 370 mm in total (KNMI). It is worth noting that this amount was higher than the average amount for this time of year, 280 mm (KNMI). For this reason, the effect of moisture on the decomposition process of the pig legs in sea soil was then studied. Temperature was not calculated in this model for the temperatures at a depth of 140 cm at this spot in the Netherlands are often low and fluctuate with ground water rising, not directly with surface temperature alterations.

### Archaeological and pedological analysis

Following the discovery of the remains, an archaeological survey was performed at the site. During this survey, the remnants of the burial pit were found. This pit was originally approximately 1.4 m deep, but only 15 cm (i.e. the bottom of the pit) could be examined in situ because the pit was almost completely destroyed by a large excavator. The average soil temperature measured at 1.4 m below the surface level was 7.8°C at the moment of excavation. The average temperature measured at the surface level was 9.2°C. The bottom of the pit was situated circa 15 cm above the groundwater level. It was not possible to measure the pH value and moisture of the pit soil reliably due to the heavy soil disturbance at the site.

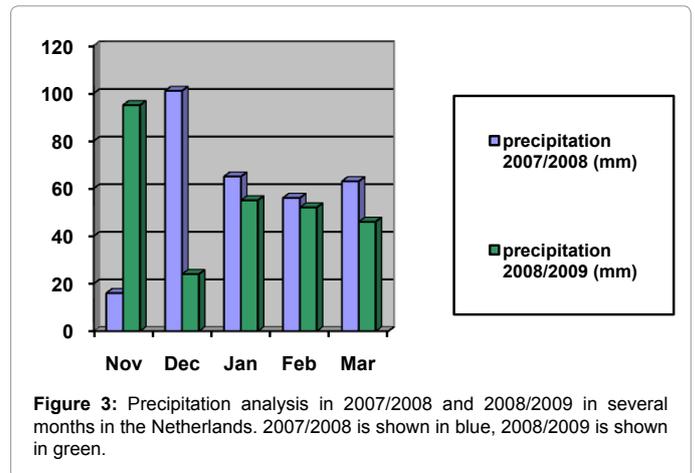


Figure 3: Precipitation analysis in 2007/2008 and 2008/2009 in several months in the Netherlands. 2007/2008 is shown in blue, 2008/2009 is shown in green.

A pedological analysis was performed in sand samples derived from the first experiment, including two sea sand samples and two woodland sand samples that were collected at the start and at the end of the entire experiment. Pedological analysis consisted of a Thermo Gravimetric Analysis (TGA), an X-ray fluorescence spectrometry analysis (XRF) and a particle size analysis 8 (Table 3 and figure 4).

### Thermo-gravimetric analysis (TGA)

The organic substance and carbonate content of the samples were determined by Thermo-gravimetric analysis. During this analysis, the loss of weight was measured in an equation with time and temperature. Data retraction took place in the range from 25 to 1000°C. All analyses were performed in duplicate. The analyses were performed on a TGA-601 machine from LECO Corporation up to 1000°C with a speed of 10°C per minute medium flow speed. We used ceramic cups and samples of  $2.5 \pm 0.4$  g.

### Rontgen-fluorescence spectrometry (XRF)

$4.5 \pm 0.005$  g samples were used in aluminium cups. 10% weight equivalent of EMU (glue substance) was added and incorporated in an Agate mill and pulverised in approximately 15 minutes. The resulting substance was added to aluminium cups and placed in a corrosion-free steel cylinder. Plastic foil was added to prevent contamination of the steel. The cylinder was placed in a press for 30 seconds with a compression force up to 2000 kg. Pressure release to 0 kg in approximately 30 seconds. Material was stored in an exsiccator until analysis. Analysis was performed with a MagiX PRO XRF system from Spectris CO. LTD. The software used was SuperQ manager.

### Grain size analysis

500 mg of sand together with 200 mg of clay was put with 5 ml of 30%  $H_2$  and boiled to remove organic material. During this heating process, extra-demineralised water was added. After cooling until approximately 40°C 5 ml 10% HCl was added before the samples where heated again for approximately 1 minute. After this, 750 ml of demineralised water was added. After sedimentation (overnight) the samples where decanted and 300 mg of  $Na_4P_2O_7 \cdot 10H_2O$  (sodium pyrophosphate) was added before heating. After cooling, the samples were analysed with a laser particle a22, C-version, VUmc, and Laboratory for Sediment Analysis.

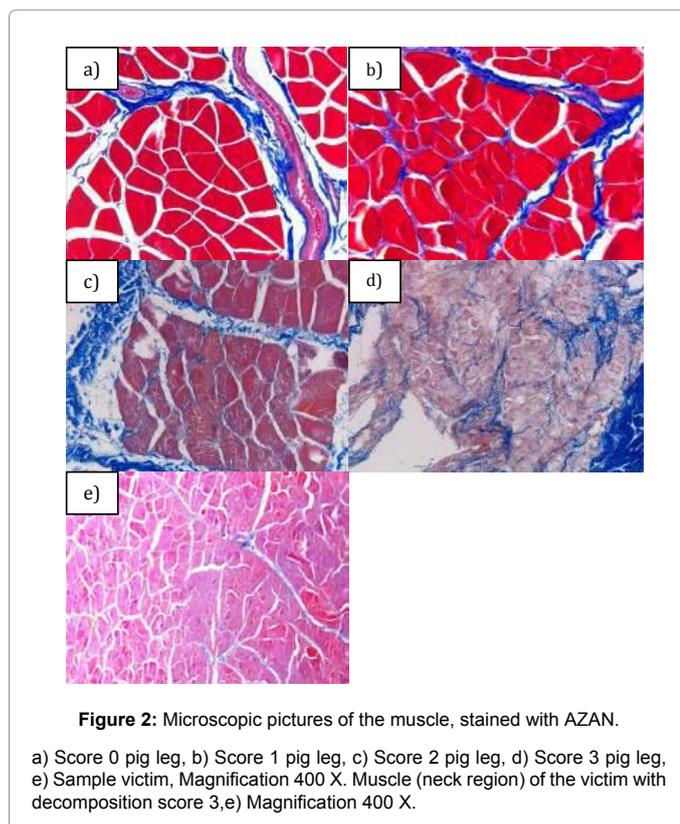


Figure 2: Microscopic pictures of the muscle, stained with AZAN.

a) Score 0 pig leg, b) Score 1 pig leg, c) Score 2 pig leg, d) Score 3 pig leg, e) Sample victim, Magnification 400 X. Muscle (neck region) of the victim with decomposition score 3, e) Magnification 400 X.

a) Results of TG analysis

Sample	Water %	Organic material %	Glow (NEN5754) %	Carbonate %
1: Woodland at start, WS	0.106	0.115	0.337	0.406
2: Sea at start, SS	0.136	0.167	0.528	5.726
3: Woodland at end, WE	0.055	0.101	0.393	0.495
4: Sea at end, SE	0.088	0.091	0.345	5.844

b) Results of XRF analysis

Spl	Conc	Fe2O3	MnO	TiO2	CaO	K2O	P2O5	SiO2	AL2O3	MGO	NA2O	Zn	Cu	Ni
WS	87,22	0,60	0,01	0,10	0,27	1,28	0,04	81,08	3,01	0,24	0,59	22,85	2,59	9,43
SS	88,48	0,62	0,01	0,12	3,69	1,24	0,04	79,03	2,75	0,38	0,59	15,03	1,59	8,29
WE	87,59	0,55	0,01	0,09	0,31	1,26	0,03	81,65	2,90	0,21	0,58	17,46	1,87	9,91
SE	87,73	0,81	0,02	0,13	5,02	1,36	0,05	75,78	3,39	0,53	0,63	18,60	2,23	10,40

Tables 3: Archeological and pedological analysis.

## Results

### Pathological analysis of the first experiment: wet sea soil versus wet woodland soil

The first study was whether significant differences were present between sea soil and woodland soil. For this pedological analysis (grain size, chemical and physical composition) was performed. Determination of the amount of organic matter in both soils showed that this was less than 0.2%. The water content of both soils at the start and the end of the experiment were also comparable, namely less than 0.14%. As expected, the only differences between sea soil and woodland soil were found in the content of calcium carbonate and in calcium oxide levels; both were 10 times higher in sea soil compared with woodland soil. Although it is known that both chemical compounds have hygroscopic potential and could therefore theoretically influence the moisture, we did not find this in our analysis (see above).

In addition, only small, but not significant, differences were found in the size of soil particles in the different soils. Namely, the sea sand showed minor reduction in grain size and a higher size variety compared to fresh woodland soil (average respectively 1.64  $\mu$ , SD 1.28 versus 1.79  $\mu$  SD 1.40).

The macroscopic aspects of the pig legs in the *in vitro* decomposition model were then analysed. No significant differences were found between legs stored in sea sand and legs stored in woodland sand at all three time points (not shown). Nevertheless, there were alterations in time between the freshly isolated material and the tissue excavated after one and three months. Namely, an increase in tissue solubility was found with increasing excavation time. This difference, however, could not be quantified for further statistical analysis.

Subsequently, putative microscopic differences were studied. As expected, the microscopic differences between fresh and unburied material were drastic. In that, all structures were optimally observed in the fresh material, whereas almost complete lyses were found in the unburied material. Microscopically, a significant increase in decomposition score was found over time ( $p < 0.05$ ) for all five layers studied (respectively dermis, muscle, fat tissue, blood vessels, nerves) between the legs buried for one and two months, as well as between the legs buried for two and three months, as depicted in figure 5. This was observed both in legs buried in woodland sand and in legs buried in sea sand. However, no significant differences in decomposition scores were detected between tissues stored in wet woodland sand and tissues stored in wet sea sand under identical temperature and identical moisture.

### Pathological analysis of the second experiment: dry sea soil versus wet sea soil

According to the first experiment, legs buried in wet sea sand showed a significant increase in decomposition scores between one as opposed two months and between two as opposed to three months (Figure 5) ( $p < 0.05$ ). It must be noted that there were differences in decomposition grade between the pig legs stored in the sea soil from experiment 1 and experiment 2. The majority of these differences in decompositions score were, however, limited to 0.5 or less and therefore not significant. The time dependent increase in decomposition score was also found in legs buried in dry sea sand. Remarkably, however, the decomposition score was significantly higher in all five layers of legs buried in dry sea sand compared to legs buried in wet sea sand. This was found in legs buried for one, two and three months (Figure 6). This therefore indicates that moisture could play a crucial role in the period of decomposition in that humidity inhibits decomposition over time in sea sand.

Although the authors recognise that a direct comparison of the results from the *in vitro* decomposition pig leg model with the complexity of a complete decomposing body of the victim can be questioned, remarkable similarities in the decomposition mode of the buried victim (Figure 2) and that of the pig legs among the different tissues were noticed. Muscle tissue of the victim mostly appeared as AZAN recognisable contours (score 2) with great similarity to wet buried pig legs after 3 months old (Figure 2e). The dermal structures showed clear lyses and collagen degeneration, while the blood vessels and nerves were well preserved with degenerated but recognisable structures and were therefore also in line with wet pig legs buried for three months (not shown). The same was true for the fatty tissue that appeared in a clear state of lyses, which was still easily recognisable (not shown). As far as this is concerned, all five analyzed tissues of the victim showed decomposition scores that were comparable with the decomposition score obtained in pig legs buried for three months in wet sea sand.

## Discussion

After death, human remains are subjected to the process of autolysis (i.e. enzymatic degradation of cells), putrefaction (i.e. degradation of soft tissues by anaerobic bacteria and fungi) and decay (degradation of soft tissues, mainly by aerobic decomposition and insects). Geoclimatic conditions, type of landscape, nature of parent soil material and the quality of the organic matter seem to dominate the speed of degradation [38]. It is generally accepted that burying does result in a

decreased rate of degradation. This decrease seems to be regulated by a reduced presence of insects, the nature of the parent soil material, a decrease in soil temperature, high soil moisture, greater burial depth and the degree of physical protection (e.g. clothing or plastic covers) [4,15,16,26,39-49].

In the present study, a case report was described of a man found to be buried in the western part of the Netherlands of which the post mortal interval was estimated at approximately one to two weeks, when in fact the man was missing for three months. Subsequently, the effect of the nature of the soil and moisture in a pig leg decomposition model was studied, as described in more detail in materials and methods. In addition, in legs buried in both sea sand (sand in which the man was buried) and in woodland sand (control sand), an increase in decomposition score over time was found, underlining the validity of the model to study the process of decomposition as such (Figures 5 and 6).

Using pedological analysis, differences between sea sand and woodland sand in chemical composition were detected, namely a level of calcium carbonate and oxide levels increased tenfold and reduced grain size in sea soil compared with woodland soil. Notwithstanding these differences, no effect on the decomposition grade was found in our decomposition model between wet sea sand and wet woodland sand (Figure 5). Although it is known that soil texture (i.e. grain size) and sand structure (i.e. compaction) can have a direct effect on the microbiological, chemical and physical reactions in the sand. Therefore on the sand's hydrological and diffusion properties [36,38,46,49,50], it has been shown that the sand type [51] and soil microbe content [50-52] are most likely to have a minor role during the early stages of the decomposition process.

Only a minor role for microorganisms in this type of soil is suggested due to the processing of the soil before using. Sea soil namely is brought up from the sea bed and left unprotected for a long time so that rain is able to wash out the majority of components. Further, the processing of the soil repeatedly disturbed the formation of solid structure. On the other hand, the woodland soil was brought up from a significant depth of several meters below the upper, biological active layer. Nevertheless, without any doubt it can be assumed that although the soil microbiological component is suggested to be of minimal influence in this case, the pre-existing microbiological presence (skin, intestines) are assumed to be of significant influence on decomposition speed. This influence is likely to occur due lower diffusion rate of decomposition gasses in water than they do in soil.

Apparently, the differences in grain composition and grain size we encountered in the present study are, in this case, not that important in the decomposition process we have studied.

It was found, however, that moisture did result in a significant

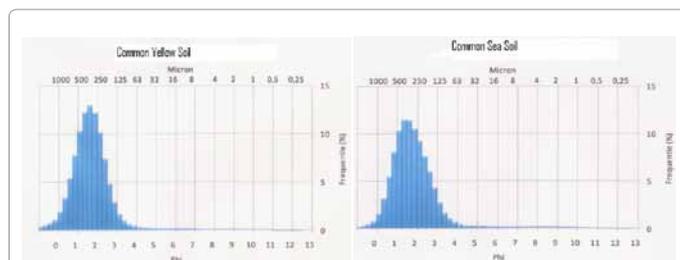


Figure 4: Grain size analysis, a) Common yellow soil, b) common sea soil.

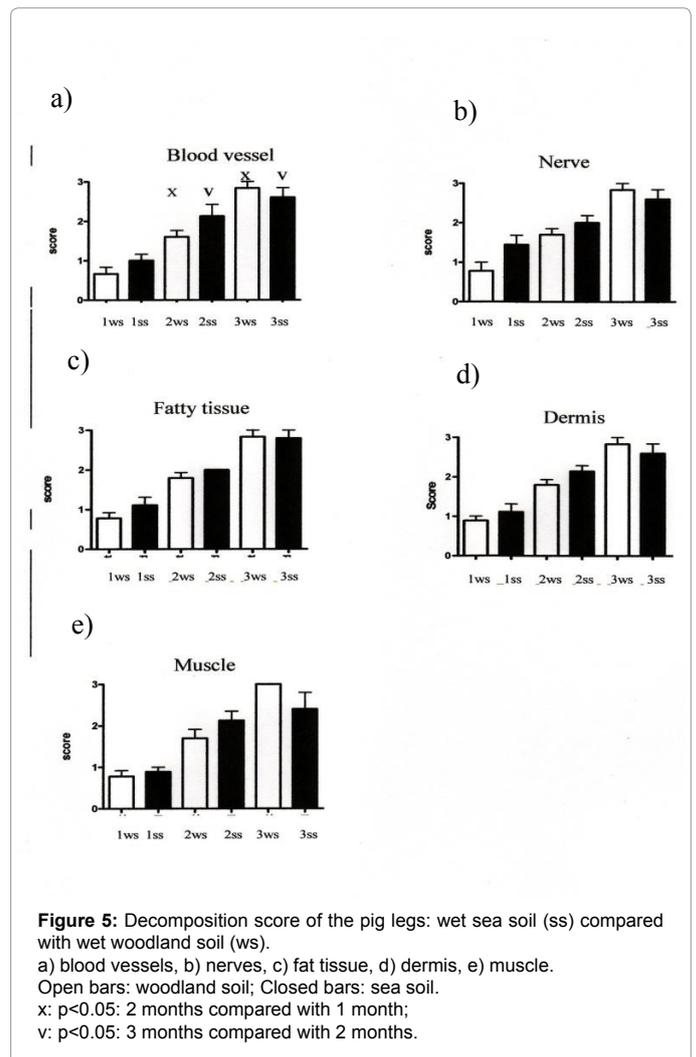


Figure 5: Decomposition score of the pig legs: wet sea soil (ss) compared with wet woodland soil (ws). a) blood vessels, b) nerves, c) fat tissue, d) dermis, e) muscle. Open bars: woodland soil; Closed bars: sea soil. x:  $p < 0.05$ : 2 months compared with 1 month; v:  $p < 0.05$ : 3 months compared with 2 months.

decrease in the decomposition score in legs buried in wet sea sand compared to legs buried in dry sea sand (Figure 5). In the literature, it was noticed that water has a preserving effect on decomposing bodies (Casper's law) [53]. The reason water preserve is likely due to the creation of a partially anaerobic environment [16].

When extrapolating the findings of the pig leg model to the particular case, it appeared that the winter of 2007 and early spring of 2008, the probable period in which the man was buried, encountered a lot of rainfall. Since the soil in the burial pit was less compact than the surrounding soil, the porosity and permeability of the soil within the pit was greater than in the undisturbed area. Theoretically, the rainwater must have therefore descended from the upper surface down into the burial pit. Furthermore, the lack of vegetation approximately the pit must have promoted the flow of rainwater into the pit. Since the remains of the victim man appear to bury only 30 cm above the water table, it is also likely that this fluctuating table were exposed to the remains. The level of the water table and the descending rainfall must have led to increasingly waterlogged soil surrounding the human remains. According to our pig leg model, it is therefore reasonable to state that the rate of degradation of these remains was slowed down.

Soil temperature can also be a denominating factor [16,45-47,49,54,55]. It is, for example, generally accepted that the degradation

of organisms and their enzymes is greatly inhibited at temperatures below 10°C [37-52,54,56-59]. During the winter and early spring months, it is known that the soil temperature in the area in which the victim was found is lower than the 8°C measured during early spring. Since the degradation of biological tissues is slowed down beneath 10°C, the low temperature in the burial pit could theoretically have played a crucial additional role in inhibiting the process of degradation of the remains in this particular case.

Another limitation of this study is that the victim was buried fully clothed while the pig samples were not. It is possible that clothing can play a role in the decomposition process. Many different types of clothing and fabrics are expected to have a deceleration influence on decomposition since they can shield oxygen and absorb moisture even in a dry period. In our model, we do not think that this effect is a major influence.

## Conclusion

In conclusion, although the authors are aware that many other influences will have different accelerating or decelerating effects on the rate of decomposition (such as clothing or the position of the body in the soil). Regarding the initial question in this case of whether the adult man could have been buried for three months, analysis of the burial pit and results from the pig leg decomposition model suggest that it is possible and, indeed, likely that the man was buried for three months in the moist sea sand.

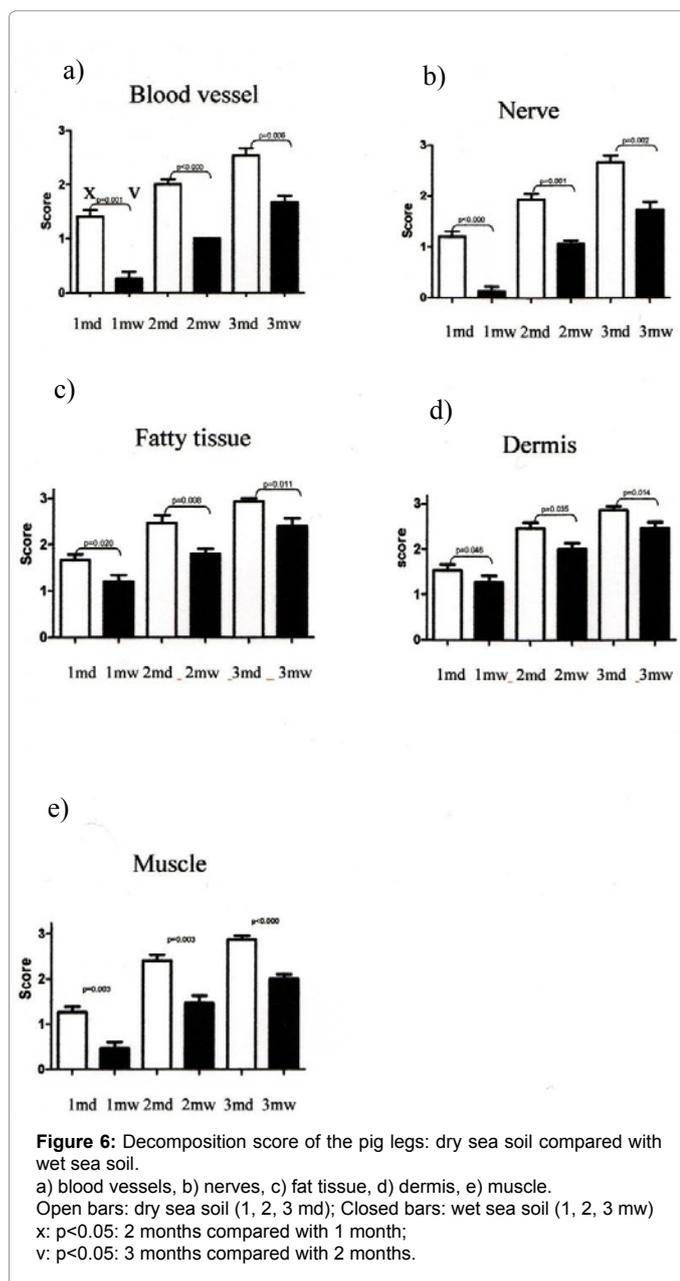
The above also shows that the burial environment is not static during the process of decomposition it will change in time. Further studies and experiments will be carried out to understand the effects of belowground decomposition on human tissues in more detail.

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