Volume XV • Issue 1/2024 • Online First

INTERDISCIPLINARIA ARCHAEOLOGICA
NATURAL SCIENCES IN ARCHAEOLOGY
homepage: http://www.iansa.eu

A Study of Species and Individual Differentiation of Burnt and Unburnt Bones Using X-Ray Fluorescence

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ARTICLE INFO

Article history:
Received: 15th March 2023
Accepted: 22nd November 2023

DOI: http://dx.doi.org/10.24916/iansa.2024.1.4

Key words:
bioarchaeology
burnt bones
individual differentiation
species differentiation
linear discriminant analysis
pXRF

ABSTRACT

Commingled remains analysis is a fundamental problem in bioarchaeology and forensic anthropology. In cases of commingling, the bones of all individuals represented must be accurately and reliably differentiated. X-ray fluorescence spectrometry (XRF) can identify species and individuals when bone fragments are found in archaeological or forensic contexts. The present study aimed to verify whether portable X-ray fluorescence spectrometry (pXRF) can be used to differentiate bone fragments at the species level (*Bos taurus*, *Sus domesticus*, *Equus caballus*, and *Homo sapiens*) and at the individual level of human individuals. We also aimed to verify whether species and individuals can be differentiated using pXRF even if the bones were burned. A total of 119 adult human bones from archaeological sites in Central Moravia and Silesia and 17 adult non-human bones from archaeological sites in Central Moravia (Czech Republic) were examined. All bones are dated to various periods (from the Bronze Age to Modern Times). When differentiating the unburnt and burnt bones at the species level, the overall accuracy of classification was 84.6% and 93.9%, respectively. When differentiating unburnt human bones at the individual level, the correct classification ranged from 88.1% to 72.7%. The differentiation of the burnt bones of humans at the individual level achieved an average success rate of more than 60%. The results confirmed that pXRF can be used for species and individual differentiation of unburnt bones and is almost equally applicable to burnt bones.

1. Introduction

It is not always easy to differentiate bone fragments at a species or individual level, especially if the osteological material is fragmented and specific features are not preserved (Adams and Byrd, 2014). Nevertheless, it is a basic requirement in funeral archaeology where burials may be represented by a cluster of secondary deposited bones (Duday *et al.*, 2009; Knüsel and Robb, 2016), and in forensic anthropology where we encounter commingled bones as a result of mass disasters (Gonzalez-Rodriguez and Fowler, 2013; Zimmerman *et al.*, 2015). An even more challenging task is the differentiation of burnt bone fragments found in archaeological and forensic contexts. The weight of burnt bones in graves tends to be highly variable throughout various time periods. In a grave, a larger number of bones is usually explained by the presence of more individuals or by their mixing with non-human bones (Wahl, 2008). For that reason, we consider it important to distinguish individual bones in the case of cremation burials.

Differentiation is not usually an issue when we encounter complete sets of remains or when anatomically characteristic features are preserved. However, many species share similar morphological structures, and when combined with bone fragmentation, it may be less easy to differentiate the species. The estimation can also be problematic in the case of juvenile bones (Dobisíková and Eliášová, 2012; Urbanová and Novotný, 2005). One option is to use microscopic methods based on the different structures of the Haversian system between species (Cattaneo *et al.*, 1999; Urbanová and Novotný, 2005). It is often not possible to apply macroscopic or microscopic methods when examining burnt bones because their shape and size change, as does the

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lamellar structure of compact bones when they are exposed to heat (Christensen *et al.*, 2012; Zimmerman *et al.*, 2015; Absolonová, 2012; Absolonová *et al.*, 2012; Ellingham *et al.*, 2015). Besides macroscopic and microscopic methods, there are molecular biological methods, usually only applicable to unburnt bones, which include immunochemical methods (Lowenstein *et al.*, 2006; Ubelaker *et al.*, 2004), DNA analysis (Bataille *et al.*, 1999; Imaizumi *et al.*, 2002), and zooarchaeology by mass spectrometry (Buckley *et al.*, 2009; Evans *et al.*, 2023). These tend to be time-consuming and costly. For burnt remains, ^{14}C dating and strontium isotope analysis are also applicable to distinguish several individuals from different time periods in the urn (Sabaux *et al.*, 2021). Another option is to use X-ray fluorescence spectrometry (XRF) or its portable version (pXRF) (Gonzalez-Rodriguez and Fowler, 2013; Christensen *et al.*, 2012), a non-destructive method of elemental analysis. The former is widely used in geology (Richter *et al.*, 2006; Ge *et al.*, 2005), archaeology (Hunt and Speakman, 2015; Smejda *et al.*, 2017), art (Calza *et al.*, 2015; Križnar *et al.*, 2011) and history (Rammlmair *et al.*, 2007; Janssens *et al.*, 2000), and has the advantage of being fast and relatively inexpensive (Šmejda *et al.*, 2018; Perrone *et al.*, 2014; Glascock, 2011).

The XRF method is based on the assumption that each species and individual have a unique elemental osseous composition that reflects the environment in which they live, the food they consume, and their metabolism (which is based on their unique mineral absorption) (Darrah, 2009). However, in the archaeological context, post-depositional processes, such as diagenesis, also play a significant role in the elemental composition of bones (Prokeš, 2007; Lebon *et al.*, 2010). The XRF method has been applied in several studies in which it was used to establish the chemical composition of material for the further differentiation of human and non-human skeletal material (Nganvongpanit *et al.*, 2017; Zimmerman *et al.*, 2015; Zimmerman, 2013; Buddhachat *et al.*, 2016; Nganvongpanit *et al.*, 2016; Christensen *et al.*, 2012), other biological materials (shells, ivory, and so on) (Zimmerman *et al.*, 2015; Zimmerman, 2013; Buddhachat *et al.*, 2016; Meizel-Lambert *et al.*, 2015; Meizel-Lambert, 2014; Christensen *et al.*, 2012), non-biological materials (glass, stone, and so on), and taphonomically-altered material (Christensen *et al.*, 2012; Zimmerman *et al.*, 2015; Zimmerman 2013; Meizel-Lambert *et al.*, 2015; Meizel-Lambert, 2014).

The pXRF method has also been employed as a tool to identify the infills of urns with and without burnt remains (Pankowská *et al.*, 2018). Gilpin and Christensen (2015) examined the presence of non-osteological contaminants in cremains and found significant changes in detected phosphorus, potassium, zinc, aluminium, and sulphur levels. Gonzalez-Rodriguez and Fowler (2013) and Perrone *et al.* (2014) were the first to apply the XRF and pXRF methods, respectively, to human osteological material from an archaeological site (Gonzalez-Rodriguez and Fowler, 2013) and from donated bodies (Perrone *et al.*, 2014), and they also investigated its potential in differentiating bones at the individual level. Finlayson *et al.* (2017) used a combination of sorting techniques, one of which was pXRF, to differentiate the remains of two individuals. Winburn *et al.* (2017) used pXRF for the differentiation of two individuals represented by commingled bones recovered from a freshwater context. According to the authors, pXRF failed to differentiate between the individuals, likely due to the diagenetic alteration of all the remains by the water environment. However, the authors did not use multivariate analysis; after recalculation of their data using LDA by the authors of this article, the differentiation between two individuals was 77.8%. McGarry *et al.* (2021) tried to differentiate individuals of five burnt and originally fresh lambs (*Ovis aries*). The bones were sectioned and experimentally burned at five different temperatures; all individuals were successfully distinguished

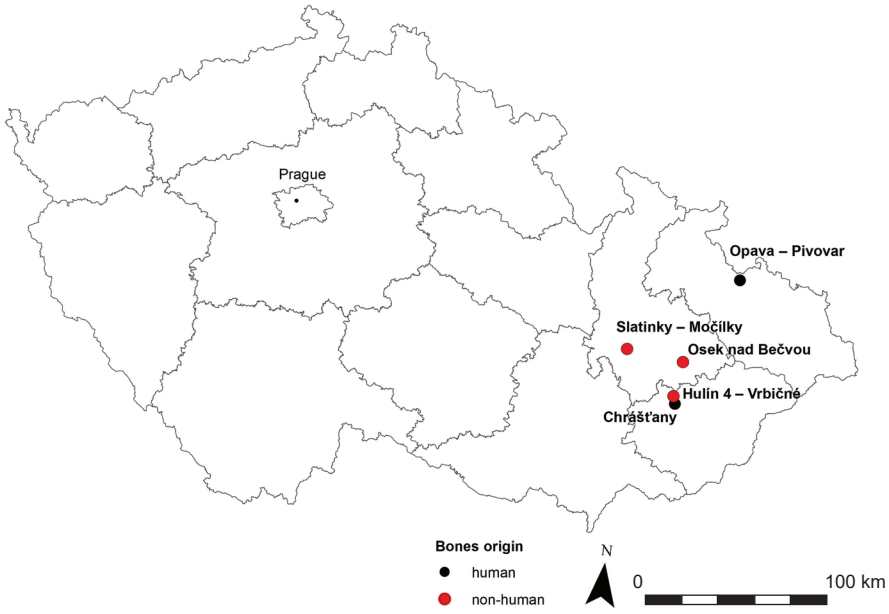

Figure 1. Location of sites within the Czech Republic.

(more than 80% of the fragments were correctly classified). Most of these studies have determined that pXRF has the potential to differentiate individuals. However, bones from archaeological contexts are affected by vital elemental exchanges in the soils surrounding them, and therefore, the classification of burnt and unburnt bones from archaeological sites can vary significantly.

The present study aimed to test the applicability of pXRF as a tool to help distinguish between bone fragments from an archaeological context at the species level (*Bos taurus*, *Sus domesticus*, *Equus caballus*, and *Homo sapiens*) as well as at the individual level for humans. We also examined whether species and individuals, from archaeological sites or elsewhere, could be distinguished after the bones had been exposed to fire.

2. Materials and methods

An analysis using pXRF was conducted on 136 human and non-human bones from five archaeological sites in Central Moravia and Silesia in the Czech Republic (Figure 1), thanks to the permission of the Archaeological Centre in Olomouc. The human bones were excavated from the Opava – Pivovar site dating to Modern Times (Dofková *et al.*, 2015) and the Chráštany site dating to the Early Bronze Age (Paulus, 2011). Non-human bones came from Slatinky – Močílky (not dated, disrupted overburden), Osek nad Bečvou dating to the Bronze Age (Tajer, 2018), and Hulín 4 – Vrbičné dating to the Bronze Age (Tajer, 2011).

A total of 119 bones of 11 human adults and 17 bones from four non-human adults were analysed (Table 1), the animals being: cattle (*Bos taurus, n*=2), a pig (*Sus domesticus, n*=1), and a horse (*Equus caballus, n*=1).

We also selected 19 bones from the sample and burnt them in an experimental cremation. The burnt sample consisted of 12 human long bones from six individuals from the site in Opava – Pivovar and seven non-human bones (long bones and two mandibles).

Table 1. The number of human (eleven individuals) and non-human (four individuals) bones.

Site	Human bones	Non-human bones
Chrášťany	45	0
Opava – Pivovar	74	0
Osek nad Bečvou	0	4
Hulín 4 – Vrbičné	0	7
Slatinky – Močílky	0	6
Total	119	17

2.1 Sample preparation

Before the analysis, three areas on each bone were selected and cleaned with acetone to remove any unwanted contamination (Moncrieff and Weaver, 1992; Pankowská *et al.*, 2018). Subsequently, selected areas were marked on the bones with a pencil to identify the area of measurement and each area was assigned a measurement sequence number.

2.2 Experimental cremation

The experimental cremation was carried out in an outdoor fireplace with natural access to oxygen and was not reloaded with more wood after being set on fire (Figure 2). The burning took place at a low temperature as temperatures of those above 700–800°C cause absolute dehydration, that is loss of water and recrystallisation of the mineral fraction of the bone tissue, which can significantly modify the chemical content of the bones (Prokeš, 2007; Castillo *et al.*, 2013). A low temperature during cremation is also assumed for the prehistoric cremations. The temperature was monitored with a Mastech MS8215 digital meter, to which a platinum sensing wire was attached. All the bones were exposed to fire for 120 minutes, and the average temperature was 712°C

Figure 2. Outdoor fireplace during the experiment.

Table 2. The number of measured points on human bones.

Site	The number of measured points		
	Unburnt bones	Burnt bones	Total
Chrášťany	129	0	129
Opava – Pivovar	205	36	241
Total	334	36	370

Table 3. The number of measured points on non-human bones.

Site	Species	The number of measured points		
		Unburnt bones	Burnt bones	Total
Osek nad Bečvou	*Equus caballus*	11	3	14
Hulín 4 – Vrbičné	*Sus domesticus*	10	6	16
	Bos taurus	7	6	13
Slatinky – Močílky	*Bos taurus*	17	6	23
Total		45	21	66

(SD 63°C). The bones were kept in the hearth until they had cooled down to prevent them from breaking. Since the temperature was low, the bones were not calcined and their colour varied from white, yellow/white, brown, grey, blue/grey to black (Ellingham *et al.*, 2015).

2.3 pXRF measurement

A pXRF spectrometer Olympus InnovX Delta from Palacký University Olomouc was used to determine the concentration of elements. The chemical analysis pXRF involves the use of X-rays. It is possible to detect elements in the magnesium Mg–U range (Palmer *et al.*, 2009; Towett *et al.*, 2016); elements with an atomic number lower than Mg are assigned as light elements (LE). The measurements were conducted in GeoChem mode which is best suited for analysing bone material (Pankowská *et al.*, 2018). Soil GeoChem mode calibrations are based on the expectation that there are many lighter elements like SiO_2 in the sample. Soil mode uses 'Compton Normalisation'. Each measurement was performed for 4 minutes using a 10 kV beam. The concentrations were given in weight percentages (Gonzalez-Rodriguez and Fowler, 2013; Perrone *et al.*, 2014).

A total of 334 and 45 points were measured on the unburnt human and non-human bones, respectively. A total of 36 and 21 points were measured on the burnt human bones and non-human bones, respectively (Table 2 and Table 3).

2.4 Statistics

To test the accuracy of the pXRF, we measured eight bones twice and examined the observational error. We compared the error between measurements using the mean difference between the first and second ones (Figure 3 and Figure 4). We verified the significance of the differences using a two-sample paired *t*-test ($p<0.05$).

To assess intra-skeletal variability, ANOVA tests were computed in individual number 129 from the Chrášťany site. Seven bones were measured, each one three times (parietal bone, radius, rib, tibia, scapula, calcaneal bone, and sternum).

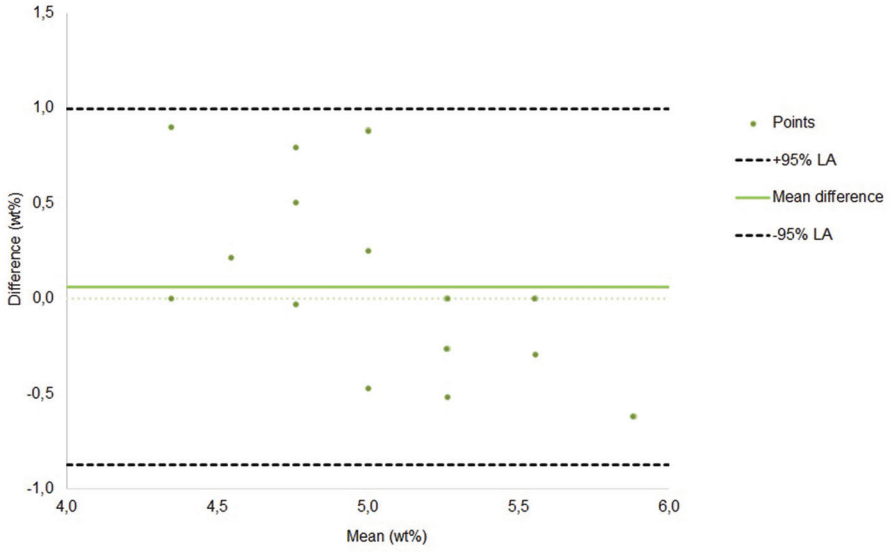

Figure 3. Bland-Altman plots depicting mean difference for points measured on selected bones.

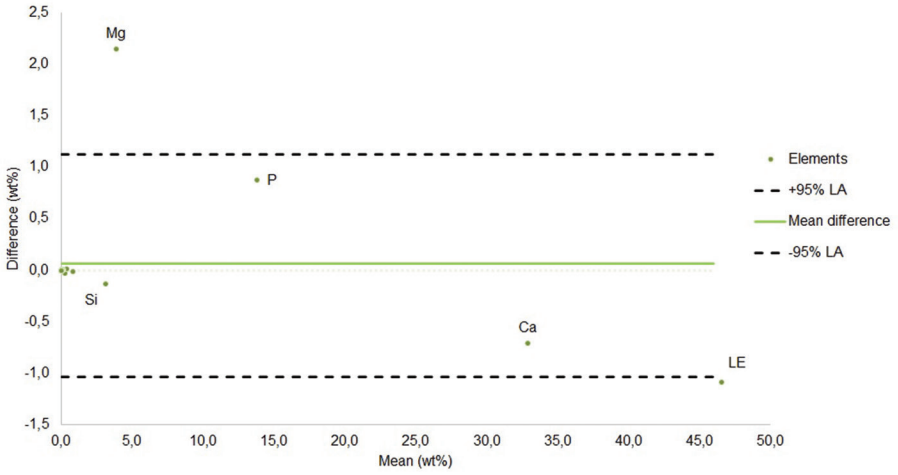

Figure 4. Bland-Altman plots depicting mean difference for elements measured on selected bones.

To discover which elements best discriminated between the single species and individuals, we used linear discriminant analysis (LDA) (Rencher, 2002; Balakrishnama and Ganapathiraju, 1998). Data were analysed and visualised with R (4.0.5) and RStudio (1.4.1106) (R Core Team 2019). We verified the fulfilment of the conditions for the use of LDA using the Mardia multivariate test (Mardia, 1970) and the Box M test (Rencher, 2002).

Since LDA is affected by the number of variables in a dataset, we removed the elements where less than half of the points were measured; since zero values would be replaced with the average measured value of the elements, this would affect the analysis. We also calculated the Wilks lambda for each element to determine the significance of each element in predicting the objects' category. If the values of the Wilks lambda were small and the elimination of that given element did not increase them above 0.3 for the whole set, the element was excluded from the analysis. We also removed elements that do not occur naturally in osteological material (*i.e.*, Zr, Ti, and U) (Gonzalez-Rodriguez and Fowler, 2013; Hedges and Millard, 1995) to distinguish the species and individuals. If unmeasured values remained for any elements, which did happen in a small part of the dataset, the program replaced them with the average measured values of the element.

3. Results

Portable X-ray fluorescence (*pXRF*) is a potentially powerful research tool for bone elemental composition analysis. The results show clear differences in chemical content among species in both the unburnt and burnt states. Unsurprisingly, individual differentiation was lower than species differentiation; however, it still achieved high values.

3.1 Species differentiation of unburnt bones

The overall accuracy of classification was 84.6% (Table 4). *Bos taurus* was misclassified twice as *Sus domesticus* and once as *Equus caballus*. *Equus caballus* was once misclassified as *Homo sapiens*. *Homo sapiens* was misclassified seven times as *Bos taurus*, once as *Sus domesticus,* and twice as *Equus caballus*. The only species that was classified correctly every time was *Sus domesticus*. There was a visible separation of individual species based on their elemental concentration. The analysis was affected the most by Zn, which affected the LD1 axis, and S, P, and Pb which affected the LD2 axis. To a lesser extent the LD2 axis was affected by Fe, Al, and Mn (Figure 4). Light elements and Si affected the LD1 and the LD2 axis.

3.2 Species differentiation of burnt bones

The overall accuracy of classification for species differentiation was 93.9% (Table 5). The only species that was misclassified was *Bos taurus*, once as *Sus domesticus* and once as *Equus caballus*. The analysis was most affected by Pb, Sr, As, and Rb which affected the LD1 and the LD2 axis, and Si which affected the LD1 axis the most (Figure 6).

3.3 Individual differentiation of unburnt human bones

Differentiation at the individual level was performed in all possible combinations according to the site the bones came

Table 4. The confusion matrix showing results for LDA of species differentiation of unburnt bones.

Species	*Bos taurus*	*Sus domesticus*	*Equus caballus*	*Homo sapiens*	**Total**	**Correct classification (%)**
Bos taurus	21	2	1	0	24	
Sus domesticus	0	10	0	0	10	
Equus caballus	0	0	10	1	11	
Homo sapiens	7	1	2	36	46	
Total	28	13	13	37	91	**84.6**

Table 5. The confusion matrix showing results for LDA of species differentiation of burnt bones.

Species	*Bos taurus*	*Sus domesticus*	*Equus caballus*	*Homo sapiens*	Total	Correct classification (%)
Bos taurus	10	1	1	0	12	
Sus domesticus	0	6	0	0	6	
Equus caballus	0	0	3	0	3	
Homo sapiens	0	0	0	12	12	
Total	10	7	4	12	33	**93.9**

Table 6. Values (%) of LDA differentiation of combinations of three, four and five unburnt individuals from the site Chrášťany and Opava – Pivovar.

Site	Three individuals		Four individuals		Five individuals	
	Mean	SD	Mean	SD	Mean	SD
Chrášťany	88.6	4.7	86.1	1.8	–	–
Opava – Pivovar	88.1	7.3	82.7	6.6	77.2	6.6

from – a combination of three, four, and five individuals all came from the Chrášťany or Opava – Pivovar site. A combination of six individuals was only performed with individuals from the Opava – Pivovar site. The correct differentiation of three individuals from the Chrášťany and Opava – Pivovar sites was 88.6% and 88.1%, respectively (Table 6). The differentiation of four individuals produced similar results. The correct differentiation of individuals

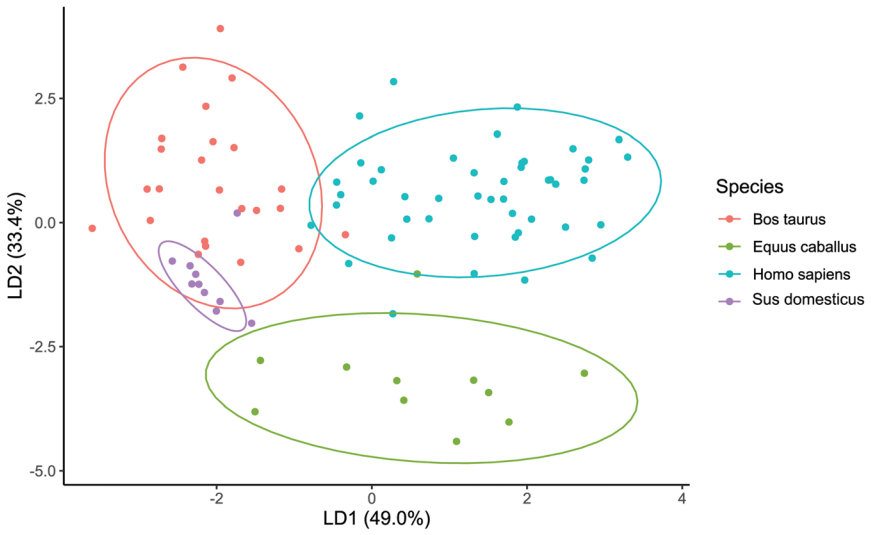

Figure 5. A linear discriminant analysis (LDA) plot showing groups of unburnt species.

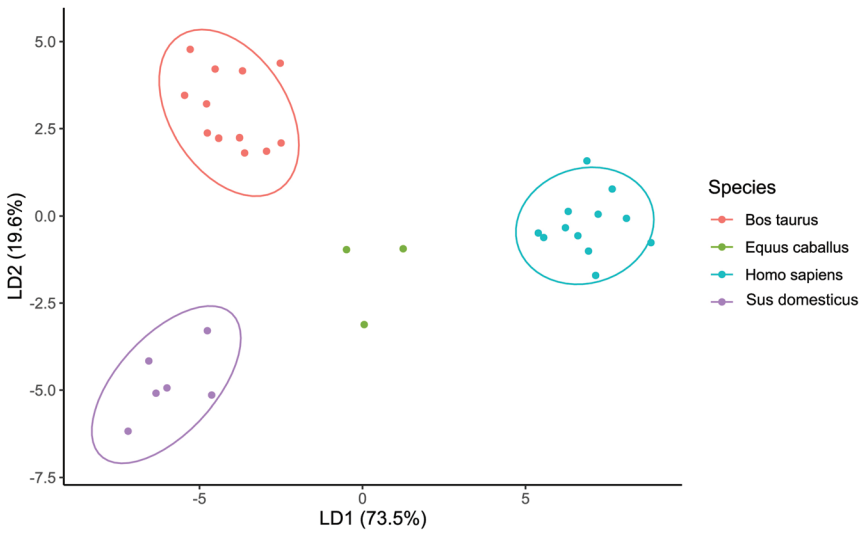

Figure 6. A linear discriminant analysis (LDA) plot showing groups of burnt species.

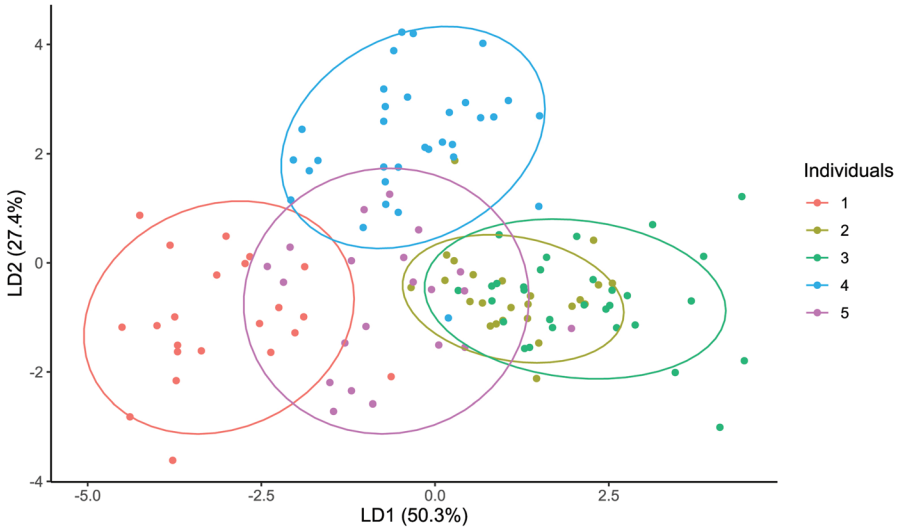

Figure 7. A linear discriminant analysis (LDA) plot showing groups of five unburnt human individuals.

Table 7. Values (%) of LDA differentiation of combinations of three, four, five and six burnt individuals from the Opava – Pivovar site.

The number of individuals	Mean	SD
Three	21.7	12.1
Four	61.7	14.3
Five	67.2	6.1
Six	63.9	24.5

from the Chrášťany (Figure 7) and Opava – Pivovar sites was 86.1% and 82.7%, respectively (Table 6). The correct differentiation of five individuals from the Chrášťany site was 83%. The correct differentiation of five individuals from the Opava – Pivovar site was 77.2% (Table 6) and 72.7% for six individuals. It was apparent that as the number of individuals increased, the differentiation became less accurate. This was probably caused by the contamination of bones due to diagenetic processes which led to a more similar chemical composition with an increasing number of individuals. Cu, Mn, Fe, S, and Zn had the greatest influence on the differentiation of individuals from the Chrášťany site, and Pb, As, Cu, Sr, and S on the differentiation of individuals from the Opava – Pivovar site. Those differences might be site-dependent since the material comes from archaeological sites and the chemical composition of bones was altered due to soil influence and diagenetic processes.

3.4 Individual differentiation of burnt human bones

The results for all possible combinations of four and five individuals achieved an average success rate of more than 60%. The overall accuracy of classification for six individuals was 63.9%. However, the analysis of the combinations of three individuals revealed an average discrimination of only 21.7% (Table 7). This might be due to the heat-inducted changes to the chemical content of burnt bones which makes them more homogenous. The analysis was most affected by Sr, Ca, and As (Figure 7).

4. Discussion and Conclusions

The rate of correct classification was 84.6% and 93.9% between the unburnt and burnt bones, respectively, at the species level. When distinguishing unburnt human bones at the individual level, the success rate for all possible combinations of three, four, five, and six individuals ranged from 88.1% to 72.7%. The discriminant analysis overall presented sufficient classification power; however, some loss can be visible with the increasing number of individuals which can be explained by the consequence of overparametrisation in the statistical analysis; *i.e.*, a smaller number of objects (individuals) with a higher number of variables always leads to better discrimination due to chance. The loss of classification power can also be associated with the increase of the chemical background noise due to soil influence and diagenetic processes. Contamination from the soil environment affecting all the bones can alter their natural similarities and differences and make it easier to alter the classification.

The differentiation of the burnt bones at the individual level achieved an average success rate of more than 60% for all combinations of four, five, and six individuals. However, the difference between the combinations of the three individuals reached an average discrimination of only 21.7%. The low discriminant power is probably affected by the heat-inducted modification which changes the chemical content of burnt bones and makes them more homogenous. Fire represents a powerful factor that erases the original differences between individuals.

Elemental concentrations slightly vary within an individual skeleton due to differences in remodelling rates, and its position in the grave. To assess whether elements vary among skeletal parts, ANOVA tests were computed in individual number 129 from the Chrášťany site. Altogether seven bones were measured, each one three times (parietal bone, radius, rib, tibia, scapula, calcaneal bone, and sternum). The ANOVA results indicate significant differences in elemental concentrations for P (F=8.9; df=6; p=0.009) and Ca (F=16.2;

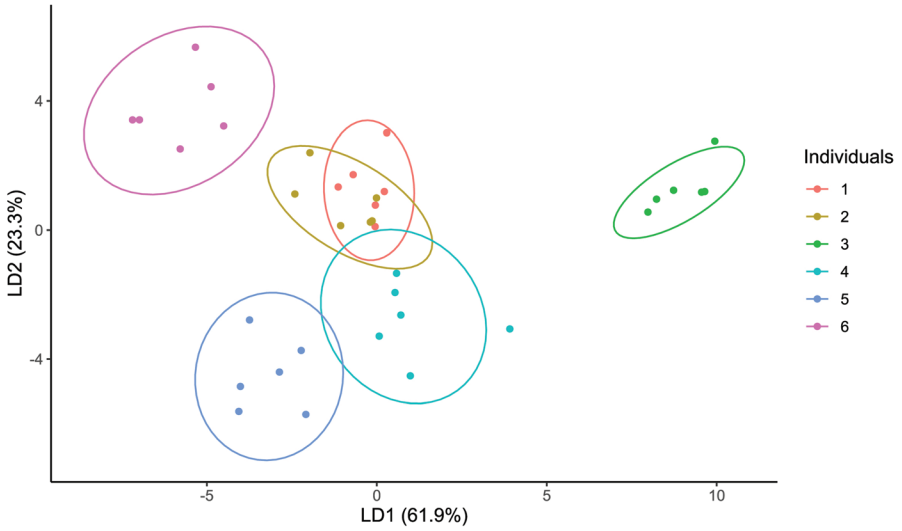

Figure 8 A linear discriminant analysis (LDA) plot showing groups of six burnt human individuals.

df=6; p=0.0001). In both cases, the calcaneal bone deviates from all the other bones. However, no intraskeletal difference was identified when the calcaneal bone was removed from the analysis. The deviation of the calcaneal bone probably reflects a variation in remodelling rates typical in the short bone, which is composed of a large part of cancellous bone (also known as spongy bone). However, when we divided the skeletal sample of human bones according to the type of bone (long, short, flat, and irregular bone), we did not record any differences. Only aluminium (Al) significantly differs among these four types of bones (F=6.9; df=3; p=0.0002). Short and irregular bones were much more enriched in Al than others. This result is not surprising since short and irregular bones are mainly formed by cancellous bone which is more susceptible to diagenetic process and elemental exchange. Concentrations of Al, K, and Mn have been shown to be more abundant in skeletons than in living individuals (Lambert *et al.*, 1985).

In our study, there was a noteworthy change in the concentrations of P, S, Ca, Mn, Fe, Zn, Sr, and Pb when species were compared before and after burning. These concentrations increased in almost all cases after burning. There was also a significant change in the concentration of Al, Si, P, S, Ca, Fe, Zn, As, Sr, and Pb when comparing individuals before and after burning. The concentrations of P, S, Ca, Fe, Zn, As, Sr, and Pb in almost all cases increased after burning, whereas the Al and Si concentrations in almost all cases decreased. This could be explained by the loss of organic matter during burning (Tofanelli *et al.*, 2014; Thompson, 2005).

In vivo chemical signatures in the bone can be obscured by postmortem changes in the soil and subsequently also by fire. However, our study demonstrates that postmortem elemental exchange inside the bone did not influence individual classification at least at the species level. According to the study of Pate *et al.* (1989), the archaeological bone is usually enriched with Si, Al, Mn, Ba, Fe, S, Sr, K, and Ti and depleted in Mg relative to modern controls. However, this is highly influenced by the particular geochemical signature of each site's subsoil and, if any such changes had occurred, it did not appear to affect the discriminant analysis. Changes in the elemental composition of the bone due to cremation do not seem as significant as one might assume. The fact that the concentration of Ca and P does not change significantly due to exposure to fire was previously stated in a study by Reidsma *et al.* (2016). According to Végh *et al.* (2022), Ca, P, Fe, Al, Si, and Sr are not significantly altered by burning. In relation to that finding, the increased concentration of Sr in our sample was surprising since other studies confirm that the Sr isotope ratio ($^{87}Sr/^{86}Sr$) (Dalle *et al.*, 2022; Harbeck *et al.*, 2011; Snoeck *et al.*, 2016) and concentration of Sr (Dalle *et al.*, 2022) is unaffected by burning. We cannot discuss the matter of $^{87}Sr/^{86}Sr$ since it was not examined in our study. However, the different results for the Sr concentration may be related to the device that was used for measuring (electron microprobe analysis, inductively coupled plasma mass spectrometer vs. pXRF) and the preparation of the samples. Our Sr concentration result may have been caused by some contamination from the fuel. During the combustion of coal and oil, the concentration of Sr increases (ATSDR, 2014), and hence it is possible that the increase in Sr values was due to the combustion of wood. Further research might focus on this issue.

Studies have shown that pXRF can differentiate bone and non-bone material. The success rates were 94% when distinguishing bone from non-bone material such as scallops, ivory, starfish, bark, a beer bottle, limestone, *etc.* (Zimmerman *et al.*, 2015; Zimmerman, 2013), and 97% when distinguishing bone from non-bone material such as float glass, oyster shell, plastic, rock apatite, starfish, wood, *etc.* (Meizel-Lambert *et al.*, 2015; Meizel-Lambert, 2014). Success rates in studies that examined species differentiation varied from 75% (Buddhachat *et al.*, 2016) to over 90% (Nganvongpanit *et al.*, 2016). These and our results show that pXRF and discriminant analysis can distinguish individuals at the species level with a success rate of over 90%. This makes pXRF a suitable tool with which to distinguish the burnt and unburnt bones of individuals at the species level.

Gonzalez-Rodriguez and Fowler (2013), Perrone *et al.* (2014), Finlayson *et al.* (2017), McGarry *et al.* (2021), and the present study show that XRF and pXRF combined with statistical analysis can be used to differentiate individual remains. However, it should be noted that: as the number of individuals increases, the success rate decreases (Table 6 and Figure 6), an effect noted by Gonzalez-Rodriguez and Fowler (2013). The loss of classification power is probably caused by the increase of the chemical background noise due to soil influence and diagenetic processes. Contamination from the soil environment can alter the natural similarities and differences of bones and make it easier to alter the classification. This means that an increasing number of individuals might lead to a more similar chemical composition for the whole sample. This indicates that differences between individuals need to be studied further. Conversely, our analysis of burnt remains showed that the success rate of classification decreased with smaller numbers of individuals. The heat-induced changes in the chemical composition of burnt bones probably cause more homogenous results and that leads to lower discrimination power (Thompson, 2004; 2005).

The advantages of pXRF have been summarised above. However, certain issues should be taken into consideration when working with XRF or pXRF. As already stated above, and by Guimarães *et al.* (2016) or Byrnes and Bush (2016), we need to consider the alteration of elements in osteological material due to diagenesis, especially when analysing archaeological material. There is a vital exchange of elements between skeletal tissue and the soil environment. Exchanges are mediated by microorganisms and significantly influenced by the geochemical composition of the soil. Each archaeological site is characterised by a particular geochemical composition which causes the separation of sites from each other. There is also the ability and/or possibility of detection of secondary radiation of low proton number elements. This is because elements with a low proton number have a low radiation signal (Kučera *et al.*, 2021; Pollard *et al.*, 2007). Additionally, the radiation can be absorbed by the air in the space between the sample and the pXRF instrument. Therefore, samples should be measured when they are in direct contact with the instrument to avoid data loss (Pollard *et al.*, 2007). Pitakarnnop *et al.* (2020) used 15 kV and 50 kV for species differentiation and concluded that combining the data generated more precise results. That is a piece of valuable information that should be reflected in future studies.

Future studies might focus on the impact of additional factors, such as the site where the remains are deposited. A separate study could be carried out in the present context, especially with regard to the discrimination of species (human individuals in particular). It might involve collaboration with a geologist and a hydrologist, who could provide data on the chemical composition of soil and water in the vicinity of the remains. Future studies might also focus on discriminating between the remains of archaeological sites and recent cases and find out whether these data are not affected by other factors that would have to be considered in subsequent analyses. Another area that should be explored further is the effect of heat on osteological material in terms of species and individual differentiation.

The present study shows that pXRF has the potential to differentiate both burnt and unburnt bones at the species and individual levels. However, further research examining the influence of sites, the ratio and concentration of elements on differentiation, and the effect of heat exposure are needed.

Acknowledgements

The authors would like to thank Lukáš Šín, Arkadiusz Tajer, and Tomáš Jelínek for providing animal and human skeletal material, as well as the staff of the Department of Geology at Palacký University in Olomouc and Lucie Formánková for measuring the elemental composition of the skeletal material using pXRF. We would also like to thank David Rieger from the New Technologies Research Centre (NTC) in Pilsen for his advice on the XRF principle and Patrik Galeta for consultations regarding the statistics used in this study. We thank the anonymous reviewers for their careful reading of our manuscript and their many insightful comments and suggestions.

This study was funded by the Internal Grant Agency of the Faculty of Environmental Sciences, Czech University of Life Sciences Prague, Grant Number: IGA 2020B0027, and Internal Grant Agency of the Faculty of Arts, University of West Bohemia, Pilsen, Grant Number: SGS-2023-002.

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