

# **Elements May Not Be Homogenously Distributed throughout The Bone, an Issue of Concern When Using X-Ray Fluorescence in Species Classification**

**Tanita Pitakarnnop<sup>1,2</sup>, Kittisak Buddhachat<sup>3,4</sup>, Promporn Piboon<sup>2</sup>, Wannapimol Kriangwanich<sup>2,5</sup>, Pongpitsanu Pakdeenarong<sup>1</sup>, and Korakot Nganvongpanit<sup>2,4\*</sup>**

<sup>1</sup>*Forensic Science and Criminal Justice, Faculty of Science, Silpakorn University, Nakhon Pathom 73000, Thailand*

<sup>2</sup>*Animal Bone and Joint Research Laboratory, Department of Veterinary Biosciences and Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand*

<sup>3</sup>*Department of Biology, Faculty of Science, Naresuan University, Phitsanulok 65000, Thailand*

<sup>4</sup>*Excellence Center in Veterinary Bioscience, Chiang Mai University, Chiang Mai 50200, Thailand*

<sup>5</sup>*Leibniz Institute for Farm Animal Biology, Wilhelm-Stahl-Allee 2, Dummerstorf, 18196 Germany*

\*Corresponding author. E-mail: korakot.n@cmu.ac.th  
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## **ABSTRACT**

*One major question that arises when we talk about the elements in bones is whether all bones contain the same elements. This study was implemented to answer this question and determine what the elemental levels are in the femur bones of pigs using the handheld X-ray fluorescence technique. Ten dry femur bones taken from adult domestic pigs (*Sus scrofa domestica*) were scanned using a handheld XRF analyzer. We compared three different groups in this study. First, comparisons were made between six locations of the whole femur bones as follows; compact bone at diaphysis (CD), compact bone at epiphysis (CE), spongy bone at diaphysis (SD), spongy bone at femoral head (SFH), spongy bone at femoral trochlea (SFT) and spongy bone at metaphysis (SM). Second, the different parts of the compact bones at the diaphysis were compared (proximal, middle and distal), Third, comparisons were made among four directions at the diaphysis (cranial, caudal, lateral and median). Differences in the elemental percentages and Ca/P ratio among all locations were determined by one-way ANOVA. The presence of most of the detected*

*elements (19 from 25) in all specimens, and that the Ca/P ratio differed significantly ( $P < 0.05$ ) when all six parts were compared. The highest percentage in all elements was observed in CD. Notably, this data is important in terms of the elements studied. We have proven that the elements were not equally distributed throughout the bones; however, there may not be any clear effects on species classification using the elemental composition in bones.*

**Keywords:** Distribution, Long bone, Element, XRF

## INTRODUCTION

Species identification is an important issue in forensic science. Species identification can be effectively achieved when complete organ samples or bone samples are used (Schotsmans et al., 2017). On the contrary, incomplete specimens of the remains of bones contribute to some of the difficulties that are associated with species identification. Currently, there are many approaches used for species identification that can deliver a high degree of accuracy. Species identification can be successfully established through the use of many of these approaches using incomplete bone samples. More specifically, molecular identification (Holland and Parsons, 1999; Moore and Frazier, 2019; Pereira et al., 2019), macroscopic identification (Corrieri and Márquez-Grant, 2019), microscopic identification (Nganvongpanit et al., 2015; Cummaudo et al., 2018; Cortellini et al., 2019; Cummaudo et al., 2019) and/or elemental composition (Nganvongpanit et al., 2016c; Nganvongpanit et al., 2017b) can all be achieved with incomplete specimens. However, each approach is associated with its own set of advantages and disadvantages. For example, the molecular technique is associated with a very high rate of accuracy. However, it requires sophisticated equipment and the expertise of knowledgeable technicians, either of which can result in increased expenses with regard to their installation and implementation. Importantly, this technique cannot be done at the scene, while the use of this method may not be appropriate in some cases or situations.

However, the specific disadvantages mentioned above could be overcome by using a number of other techniques. These alternative methods can serve as screening tools that can be employed prior to the results being confirmed with a proper molecular technique. Our previous studies demonstrated that the use of elemental composition in dense connective tissues, such as the bones or teeth of humans and/or the bones, teeth or horns of animals, could effectively be used in species classification with a satisfactory degree of accuracy and a positive precision rate (Castro et al., 2010; Buddhachat et al., 2016a; Nganvongpanit et al., 2016a; Buddhachat et al., 2016b; Nganvongpanit et al., 2016b; Nganvongpanit et al., 2016c; Buddhachat et al., 2017; Nganvongpanit et al., 2017a; Nganvongpanit et al., 2017b). However, many other studies must be undertaken before it can be established that this method could be effectively used