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ANCIENT BILE ACIDS IDENTIFIED BY HPLC-ES-MS/MS IN SOIL/FAECAL SAMPLES, COLLECTED IN A NEW ARCHEOLOGICAL SITE OF SUSPECTED ROMAN SEWER AND LATRINES IN POMPEII RUINS

E. Porru[1], S. Turroni[2], M. Candela[2], R. Helg[3], E. Giorgi[3], A. Martellone[4], J. Fiori[1], A. Roda[1]

[1]*Department of Chemistry "G. Ciamician", Alma Mater Studiorum University of Bologna, Italy*
[2]*Unit of Microbial Ecology of Health, Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy*
[3]*Department of History and Cultures, University of Bologna, Bologna, Italy*
[4]*Archeologic park of Pompeii, Pompeii (Naples), Italy*

Among the different markers used in archaeology and related disciplines for detection and source identification of faecal matter in soil [1], steroid analysis is a promising tool when the archaeological context regards the potential presence of faecal sample, under oxygen-deficient conditions like in latrines or sewer system. Steroids are stable molecules well preserved and therefore used as biomarkers for a faecal input that occurred even in ancient era of thousands of years ago [2,3]. Particularly neutral steroids such as stanols and stanones (Δ5-sterols) can be used for the presence of faeces and faecal inputs into soils and sediments [4]. On the other hand, the ubiquitous occurrence of Δ5-sterols (dead plant or animal material, root exudates, faeces, or soil micro- flora and fauna), as well as their transformation to stanols in the environment, reduce their specificity as mammalian faecal biomarkers. The commonly used steroids still do not take advantage of the full potential of the steroid spectrum, because they do not consider bile acids (BAs). BAs are likely the most specific steroids markers for a faecal input, due to their exclusive occurrence in vertebrate faeces. Furthermore, BAs are more resistant to degradation than Δ5-sterols, stanols, and stanones [5] and can therefore still reveal an ancient input into soils where other markers have already been degraded or further metabolized. BAs are acidic steroids synthetized in the liver from cholesterol, are excreted into the intestine and then transformed to secondary BAs by intestinal microflora. In the human body most of the secondary BAs like lithocholic acid (LCA) and deoxycholic acid (DCA) are excreted in faeces. Due to different BA synthetic pathways and metabolism, BA profiles of vertebrates (including humans) may differ significantly [6].

Secondary BAs undergo further several metabolic pathways by gut microbiota to produce epimers and oxidized derivatives. Oxo bile acids (oxo-BAs) are present in faeces at levels similar to those of their metabolic precursor, up to 20-30 % of the total faecal BA [7]. Among mammals, faecal BA composition is therefore specie-specific and their qualitative profile can be used as powerful specific tool to identify the source of faecal samples and verify possible cross-contamination. We have developed and optimized an HPLC-ES-MS/MS method for the analysis of up to 21 BA and their oxo-BAs metabolic products in faeces. Efficient solid phase extraction and concentration procedure have been developed for BA extraction from soil

with high recovery, in order to obtain detectably concentration when the original faeces are dispersed and diluted in the soil sample of sewer and latrines. The present method was accurate (bias%<15%), precise (CV%<10%) and sensitive (LOD<30ng/ml). The MRM detection mode provided high selectivity and minimized matrix effect

This method was applied to Pompeii's soil samples from a latrine pit of a toilet, collected during recent archeological excavations in Obellio Firmo's House, to assess the presence of fecal matter.

The main BA identified in the samples were deoxycholic acid and lithocholic acid with a mean concentration of 54 ng/g and 12 ng/g respectively. The BA profile and the ratio between BA and Oxo-BA levels (about 3:1) has been used to assess the human origin of the feacal input in the archeologic samples.

The results from the analyzed samples confirm the discovery of a toilet site in Pompeii ruins, assessing the human origin of the fecal material. To our knowledge, it is the first time that Oxo-BA metabolites have been identified and measured in ancient samples.

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