Sex determination of human remains is of great archaeological significance, as it provides a more complete picture of social and familial structures within ancient societies. Typically performed through examination of bones in the pelvic region, accurate sex determination can be exceedingly challenging in the absence of a sufficiently preserved skeleton. Here, a method for sex determination in living humans, involving measurement of magnesium, strontium, sulfur, and zinc in head hair along with multivariate statistics, was applied for the first time to hair collected from 500-year-old mummies originating from Peru. Using mummy hair as the model enabled accurate sex prediction of the mummies, showing that, despite the age of the hair, the samples still contain the necessary elemental information for sex determination. For accurate sex determination of mummies using hair collected from living humans, magnesium had to be replaced by sodium due to significant differences in dietary habits. With this simple modification, hair from living humans in North America could be used to successfully predict the sex of individuals who lived more than 500 years ago in Peru. This work paves the way for broader use of non-skeletal sex determination methods within the field of archaeology, filling a significant gap.