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RESEARCH ARTICLE

Metals in bones of the middle-aged inhabitants of Sardinia island (Italy) to assess nutrition and environmental exposure

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Abstract
Metals in bones of 72 subjects lived between the twelfth and eighteenth century AC and collected in four Sardinian (Italian insular region) burial sites (Alghero, Bisarcio, Geridu, and Sassari) were determined and used as biomarkers to evaluate diet and potential social-environmental differences. Concentrations of Ba, Ca, Cd, Cu, Hg, Pb, Sr, and Zn were quantified in different types of compact bone (femur, fibula, humerus, radius, tibia, ulna) by sector field inductively coupled plasma mass spectrometry previous acidic digestion and differences among the various burial sites, centuries, types of bone, gender, and age were explored by univariate and multivariate analyses. Results indicated differences between sites in terms of diet: Bisarcio (inland village) had increased ratios of Ba/Ca and Zn/Ca due to higher incidence of vegetables, cereals, and animal foods in the diet; Geridu (coastal village) showed increased Sr/Ca ratio indicating foods of plant and marine origin that were predominant; Alghero (coastal site) and Sassari (inland site) displayed prevalently a mixed diet reflecting a higher economy and food imports. In addition, these latter sites showed increased levels of Hg/Ca (fish, drugs, cosmetics) and Pb/Ca (coins, utensils, pipeline for water). In conclusion, the elemental Ba/Ca, Sr/Ca, and Zn/Ca ratios were indicative of provenance and diet, while Hg/Ca and Pb/Ca ratios were associated to various forms of environmental exposure.

Keywords Bones · Diet · Environment · Metals · Middle age · Sardinia

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Introduction

The quantification of metals in bones of ancient populations is an attractive way to reconstruct their diet and lifestyle habits and environmental conditions considering that bones are very good biomarkers of long-term metal exposures (Allmäe et al. 2012; Stipisic et al. 2014; Zapata et al. 2006). The most studied metals to describe the diet were Ba, Cu, Sr, and Zn. In particular, Ba and Sr were considered as indicators of consumption of vegetables, plants (cereals and legumes), and marine foods (mollusks and crustacean), while Cu and Zn represented the markers of a protein diet of animal origin (red and white meats and milk products) (Allmäe et al. 2012; Bacci and Bartoli 2015; Lambert et al. 1984). Many studies found high levels of Sr and Ba and low levels of Cu and Zn as typical patterns in vegetarian and marine diets (Allmäe et al. 2012; János et al. 2011; Schutkowski et al. 1999; Stipisic et al. 2014). The measurement of Ca, as the main element of the hydroxyapatite matrix, is useful to assess the *post-mortem* preservation of bones, and it is in the 25–35% interval in good preserved bones (Allmäe et al. 2012), while the determination of elements as Cd, Hg, and Pb in bones may reflect various sources of environmental exposures like cosmetics, painting, medicine, coins, utensils, and water supply pipes (Kim et al. 2017; Nriagu 1996). Moreover, in analogy with the present day, another environmental source of Hg in the medieval time was fish in which this metal is biomagnified as methylmercury (Rasmussen et al. 2013).

In archeological studies, analysis of metals in bones aimed at assessing diet and lifestyle habits of ancient populations, the *diagenesis* of archeological bones can compromise the credibility of this information (Martínez-García et al. 2005; Nielsen-Marsh and Hedges 2000; Zapata et al. 2006). *Diagenesis* is a series of changes in the soil, including dissolution, precipitation, crystallization, and exchange of minerals between the soil and bones. According to the literature, archeological sites that are located on gentle hills and not being exposed to groundwater are less exposed to the influence of diagenesis (Nielsen-Marsh and Hedges 2000); the alkaline soil can preserve bone far better than the acid one, which easily destroys hydroxyapatite (Hedges 2002); compact cortical bone is less exposed to diagenesis than trabecular bone (Carvalho et al. 2004; Sillen and Kavanagh 1982; Ezzo 1994); the use of the ratio metal/Ca can help to minimize the impact of diagenetic processes based on the principle that it affected both Ca and trace metals in the same way (Giorgi et al. 2005; Scattarella et al. 2002). In any case, it is generally believed that factors influencing diagenesis are complex and interdependent covering geochemical, physical, and biological processes, and the quantitative analysis of diagenesis is still at a very early stage (Wilson and Pollard 2002).

In this study, 72 middle-aged human compact bones from four burial sites (Alghero, Bisarcio, Geridu, and Sassari) located in the northwest of the Sardinia island (an Italian region) were analyzed for Ba, Ca, Cd, Cu, Hg, Pb, Sr, and Zn levels to assess nutritional, social, and environmental differences. The protocol used tried to minimize possible diagenetic biases in order to offer, at best, an estimation of the elemental uptake in the lifetime of individuals. Differences between the various locations of burial sites, centuries, types of bone, gender, and age were explored. Univariate and multivariate analyses were applied to have a more complete understanding. It is the first time that a study on bone element levels has been conducted on Sardinian medieval skeletons.

Materials and methods

Burial sites

The four sites, namely, Alghero, Bisarcio, Geridu, and Sassari, are located in the northwest side of Sardinia island (Sassari Province) as shown in Fig. 1. In the middle age, this side of the isle was called "Giudicato di Torres," a state sovereign and independent, divided in various regions or "curatorie." The origin of Alghero dates back around the early twelfth century by the Genoese family of the Doria. The village was founded in the proximity of a large natural harbor, crucial to the promotion of commercial traffics with Genoa and Marseilles. The human remains were found in the medieval San Michele cemetery, very close to the homonym church, characterized by a space planning for an individual burial excavated in rock. The first group of remains dated back thirteenth to fifteenth century AC. Some elements of personal ornament, clothing, and coins would seem to report this phase and sepulchral space to a group of individuals socially privileged or connoted in a socially elevated way, a hypothesis that seems to be supported by the burial in wooden for some deceased. The second group of bones (dated sixteenth century AC) was found in long (5–6 m) and narrow burial tombs (trenches) excavated in the ground, each containing the remains of 10–15 individuals on average died at the same time or at short intervals to allow simultaneous burial. The reason of this kind of burial might be traced back to the plague epidemic that struck the city in late 1500. At present, the city that faces the sea has ca. 40,000 inhabitants representing the fifth of the whole region. The site of Bisarcio is for the Sardinian middle age a place of particular significance, being the venue of an important and ancient diocese (St. Antioco), where the human remains were excavated and dated fourteenth to eighteenth century AC. An important center, the capital of the homonym "curatoria," was placed on the road connecting the north to the south of the island. The site is ca. 50 km from the sea and of the ancient village are nowadays present only ruins located around the St. Antioco church. In this site, the burial tombs were simple and excavated directly in the rock. On the tombs were present worked stones that could be identified as a kind of tombstones to indicate the social status of the buries. Geridu is the most important archeological site of the "curatoria di Romangia" with the presence of the St. Andrea church where bones were found. During the middle age, the farmer's village was involved in commercial traffics as evidenced by some ceramics found in the burial site produced in various Mediterranean areas as Spain, north Africa, and other Italian regions (Liguria, Tuscany, and South Italy). The ruins of the ancient village are placed at north of Sassari, at 5 km from the sea. All human remains (fifteenth century AC) come from simple graves, and the decomposition environment is in the ground. The site was probably a privileged place, because bones were found adjacent to the apse where usually religious and high-class family members were buried. Sassari was probably founded around the twelfth century and soon became the most populous center of the "Giudicato di Torres" (about 10,000 inhabitants, thirteenth century) where intense commercial and artisan businesses developed. Sassari was an important

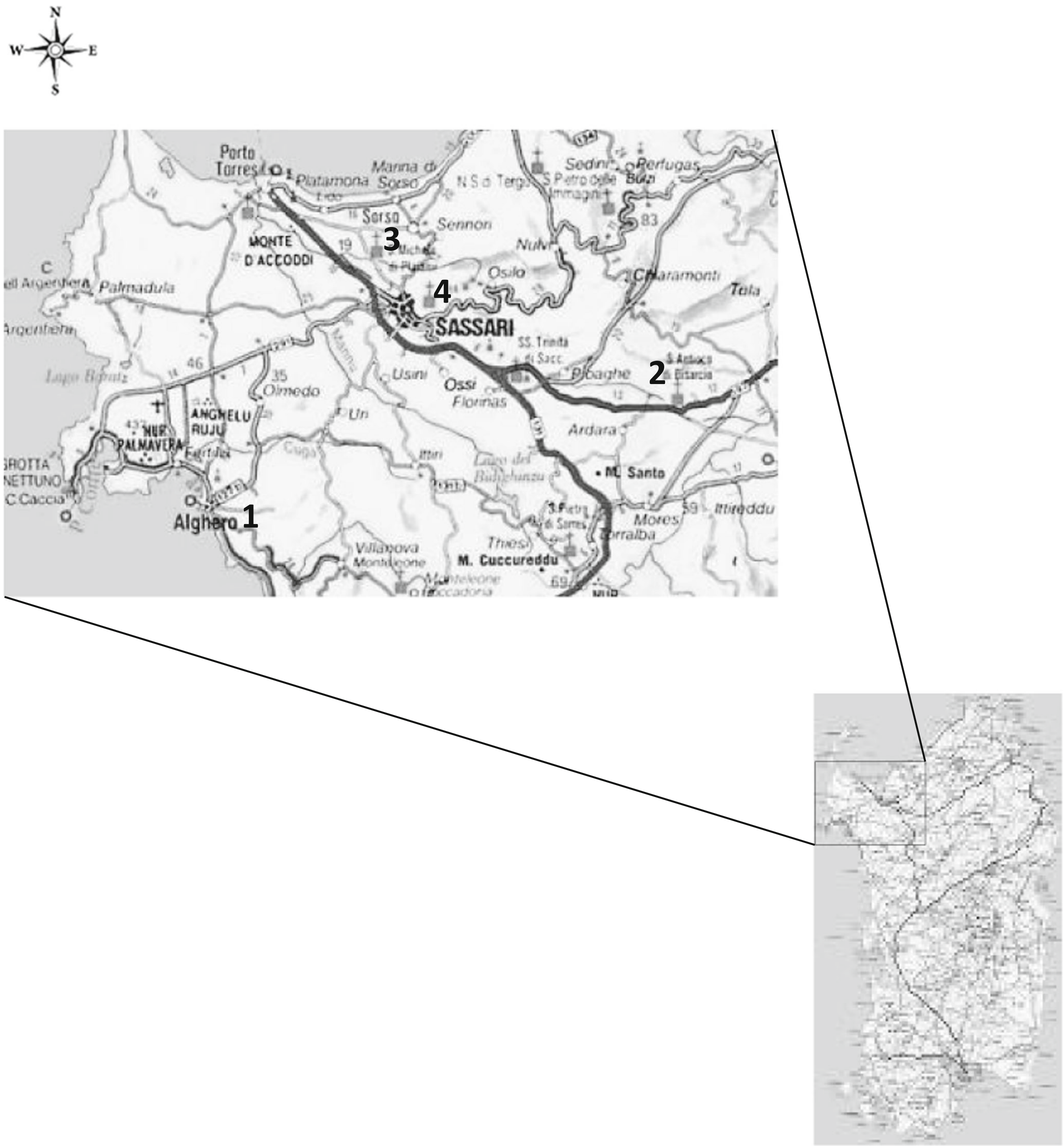

Fig. 1 Sampling areas. 1 = Alghero; 2 = Bisarcio; 3 = Geridu; 4 = Sassari

diocese with the presence of the San Nicola Cathedral, place where the bones, dated twelfth to fourteenth century AC, were recovered. The tombs were excavated directly in the rock, sometimes as single burial or often multiple burials. The city, nowadays, is the capital of the homonym province, it is far ca. 10 km from the sea, and it has ca. 125,000 inhabitants (the second city of the region) (Amadu 2003; Milanese et al. 2000; Milanese et al. 2009; Rovina and Fiori 2013).

Samples and analysis

Seventy-two fragments of long compact bones (femur, fibula, humerus, radius, tibia, and ulna) were recovered during excavations in four different burial sites in the north of Sardinia (an Italian island) (Fig. 1). In particular, 27 samples in Alghero, 14 in Bisarcio, 15 in Geridu, and 16 in Sassari were collected. Archeological evaluations have been performed to make a hypothesis of century (between twelfth and eighteenth century AC), sex (39 males and 33 females), and age (from 2–4 to 60–70 years old). Archeological information included materials (ceramics, coins, dresses), animal and plant life found during excavations, as well as the examination of religious and social registers. The bone samples corresponded to the remains of persons who died and were buried in the four areas, and who we considered probably lived most of their life in these areas. The number of samples found in the four sites divided by sex, age, and type of bone are reported in Table 1. Once collected, to remove the deposited soil, bones were gently dry-brushed, rinsed with high purity deionized water, and brushed again. Further, to remove all the embedded soil particles, samples were posed in plastic tube with deionized water and placed in an ultrasonic bath for 15 min. The cycle was repeated until the water remained clean. Then, samples were added with a 5% glacial acetic acid solution and ultrasonicated for 15 min until the solution was clean. Subsequently, samples were left overnight in 5% glacial acetic acid solution to eliminate the diagenetic soluble carbonates and the first superficial layers, potentially contaminated by contact with soil or metallic tools. Once rinsed with high purity deionized water, samples were desiccated in a drying oven at 60 °C until constant weight and stored in plastic containers. Prior the dissolution, samples were then thoroughly powdered in a ceramic mortar in a class-100 clean room with laminar flow to minimize the metal contamination.

For the digestion, ca. 150 mg of pulverized bone was weighed into a 15-ml polystyrene tube, added with 2 ml of nitric acid of ultrapure grade (VWR, Leuven, Belgium) and put into an heather block (ModBlock, CPI International, Santa Rosa, USA) at 75 °C until complete dissolution. Then, samples were diluted at the volume of 10 ml with high purity deionized water and stored at 4 °C until the analysis.

Table 1 Characteristics of the four population

	Alghero	Bisarcio	Geridu	Sassari
Total population	27	14	15	16
Sex				
Females	13	5	7	8
Males	14	9	8	8
Age				
≤ 24 years	4	10		16
> 24 years	23	4	15	
Bones				
Femur	8	4	1	8
Fibula	4		11	
Humerus	1	10	2	3
Radius	1		1	2
Tibia	9			3
Ulna	4			

The quantification was performed through the use of the sector field inductively coupled plasma mass spectrometry Element II (Thermo Fisher, Bremen, Germany). Cadmium at mass 114, Hg^{202}, and Pb^{208} were quantified in low resolution (M/dM = 300), whereas Cu^{63}, Zn^{66}, Sr^{86}, and Ba^{138} in medium resolution (M/dM = 4000) and Ca^{44} in high resolution (M/dM = 10,000). The addition calibration approach was used to quantify the metals, and Indium (mass 115) was used as internal standard at the level of 1 ng/ml in solution. The method repeatability was better than 10% and accuracy, calculated on the standard reference material no. 1486 Bone meal (NIST, Gaithersburg, USA), which was in the interval 2.4–8.7%.

Control of diagenesis

Even if some authors reported that elements (as Pb, Ca, Sr, and Zn) are good indicators of *intra-vitam* exposure as they are not subjected to *post-mortem* alterations, we have attempted to minimize the influence of diagenesis which could alter the original elemental bone composition in order to attribute the observed metal differences to distinctions between individuals during their life and not after death (Carvalho et al. 2004; Nielsen-Marsh and Hedges 2000). Regarding the type of soil, its calcareous origin in the four burial sites—with pH values slightly alkaline (between 8.05 and 8.35)—contributed to the preservation of human remains. Moreover, we analyzed Ca level in all bones, and it was constant (namely, 24–25%) between all individuals and also not different between sites (Table 2). Due to these stable Ca values, relevant phenomena of enrichment or depletion due to diagenesis can be excluded (Giorgi et al. 2005; Scattarella et al. 2002). Moreover, Ca concentration was used to normalize all metal values so as to create standardized values for better comparing dietary habits, geographical areas, and ages (Table 3). In addition, compact bones were preferred to spongy bones because of their minor susceptibility to diagenesis; also the compact bones remodeling is slower than in spongy bones, and, hence, they offered the more realistic proxy for long-term study (Sillen and Kavanagh 1982; Ezzo 1994). Moreover, considering that Radosevich (1993) attributed high values (> 1000 μg/g) of bone Sr to diagenesis, we found median concentrations (Table 2) between 250 and 622 μg/g, and these data could be indicative of a good bone preservation. Another argument against diagenesis was the significant correlation ($p = 0.008$; $\rho = 0.310$) between the Sr/Ca ratio and Ba/Ca ratio in all individuals indicating that these ratios reflected intact biological values (Burton et al. 1999).

Statistic

Since data were not normally distributed, results were expressed as median (P50) and 10th–90th percentiles (P10–P90) for metals and metals/Ca ratios. Differences in metals/Ca

Table 2 Elements concentration in bones collected at four archeological sites, expressed as median and 10th–90th percentiles in brackets

	Ba (μg/g)	Ca (mg/g)	Cd (μg/g)	Cu (μg/g)	Hg (μg/g)	Pb (μg/g)	Sr (μg/g)	Zn (μg/g)
Alghero	54.4 (29.5–97.9)	250 (204–285)	0.18 (0.10–0.64)	10.7 (6.69–22.7)	0.42 (0.17–1.67)	12.8 (8.06–28.7)	293 (167–379)	250 (208–338)
Bisarcio	183 (131–273)	243 (207–270)	0.22 (0.12–0.40)	18.9 (11.0–28.6)	0.13 (0.03–0.32)	6.04 (2.95–14.1)	265 (213–334)	350 (225–605)
Geridu	37.5 (33.3–60.4)	239 (196–266)	0.16 (0.11–0.27)	10.7 (6.90–13.4)	0.15 (0.06–0.62)	5.02 (2.52–22.4)	622 (395–944)	232 (176–353)
Sassari	29.3 (20.0–52.1)	256 (217–293)	0.27 (0.16–0.58)	34.6 (14.8–62.2)	0.74 (0.14–1.33)	14.8 (3.91–22.8)	250 (206–381)	281 (196–342)

ratios among the four population groups, inter-metal correlations, and associations with variables as sex, age (≤ 24 years, > 24 years), location (Alghero, Bisarcio, Geridu, and Sassari), and type of bone (femur, fibula, humerus, radius, tibia, ulna) were tested by non-parametric tests (U Mann–Whitney and Kruskal–Wallis), and p values < 0.05 were considered statistically significant. The hierarchical cluster analysis (CA), linear discriminant analysis (LDA), and principal component analysis (PCA) were performed using the measured metals/Ca ratios. The hierarchical CA is computed on the distance matrix using the average linkage method; it was done with the aim to arrange subjects in clusters where the degree of association is strong between members of the same cluster and weak between members of different clusters. The LDA classification model was performed using the prior probability based on the group size in order to assign each individual to a class according to burial sites. The PCA was performed using the Kaiser normalization with a varimax rotation, and only factors with eigenvalues > 1 were considered. The IBM SPSS Statistics 24 was used as statistical package.

Results and discussion

Metal levels in bones

Bones can be considered bioarchives of elements as Ba, Zn, Sr, and Pb and can contribute to answer to questions about diet habits, social aspects, working activities, and exposure to environmental contaminations of ancient human populations. We applied a multi-element approach analyzing metals (as Ba, Sr, Cu, and Zn) most likely linked to diet and others mainly correlated to anthropic activity and lifestyles (as Cd, Hg, and Pb). The absolute values of each metal found in the 72 bone samples collected at the different sites are reported in Table 2 as P50 and P10–P90. In Table 3, median results of metals/Ca ratios and log Ba/Sr values found in bone samples are reported. The Kruskal–Wallis test applied to the results of Table 2 indicated that Ba ($p < 0.009$), Cu ($p < 0.001$), Hg ($p < 0.001$), Pb ($p = 0.003$), Sr (p < 0.009), and Zn ($p = 0.017$) were statistically different among the four archeological areas. Also considering the ratio values (Table 3), Ba/Ca ($p < 0.001$), Cu/Ca ($p < 0.001$), Hg/Ca ($p < 0.001$), Pb/Ca ($p = 0.006$), Sr/Ca ($p < 0.001$), Zn/Ca ($p = 0.035$), and log Ba/Sr ($p < 0.001$) were statistically different among excavation sites. As regards Ba and Sr, these metals have been considered as indicators of consumption of an herbivorous diet, since these elements are highly concentrated in plants. Barium was also associated to consumption of nuts, berries, and cereals, and Sr to mollusks and crustaceans in the daily diet. Regarding Sr, typical concentration levels of Sr for mammalian bones by different mode of nutrition have been reported as follows: 400–500 μg/g for herbivores; 150–400 μg/g for omnivores; 100–300 μg/g for carnivorous (János et al. 2011). Other references reported Sr/Ca ratio was lower in the carnivore than in the herbivore; thus, higher values of Sr/Ca point to a vegetable rich diet (Giorgi et al. 2005). We found that, Ba concentration and Ba/Ca ratio were three to seven times higher in Bisarcio than in the other sites, while Sr value and Sr/Ca ratio were ca. 2.5-fold higher in Geridu respect to the remaining sites. These results showed that bone Ba/Ca and Sr/Ca ratios were able to distinguish individuals living in Bisarcio and consuming mainly animal-based diet form those living in Geridu consuming a vegetable-

Table 3 Median element ratios in bones collected at four archeological sites

	Ba/Ca	Cd/Ca	Cu/Ca	Hg/Ca	Pb/Ca	Sr/Ca	Zn/Ca	Ba/Sr	Log Ba/Sr
Alghero	0.20	0.001	0.05	0.002	0.06	1.11	1.05	0.18	− 0.75
Bisarcio	0.73	0.001	0.08	0.001	0.03	1.15	1.52	0.64	− 0.20
Geridu	0.18	0.001	0.02	0.001	0.04	2.61	1.02	0.07	− 1.17
Sassari	0.11	0.001	0.14	0.003	0.05	1.01	1.04	0.12	− 0.93

rich diet. Similarly, other authors used Sr/Ca and Ba/Ca ratios found in compact medieval bones of Danish subjects to differentiate individuals that lived in different geographical locations and also individuals who lived in a location for the entire life from those lived only a period of their life in the site (Rasmussen et al. 2013).

Our results indicated that individuals from Bisarcio consumed prevalently plants and cereals in their diet, while people from Geridu consumed vegetables integrated by marine foods. Moreover, since Sr is related to the consumption of marine products, the log Ba/Sr ratio can be used to estimate the proportion of marine to terrestrial products in the subjects' diet; if the ratio is more negative, the diet is more shifted to marine nutrition (Burton and Price 1990). Our results (Table 3) showed that Geridu had a log Ba/Sr of − 1.17 confirming that the diet of this area spanned towards the marine proteins, while in Bisarcio population, the log Ba/Sr of − 0.20 suggested that proteins of terrestrial origin were the main components of the diet. Differently, the log Ba/Sr values for Alghero and Sassari were intermediate (− 0.75 and − 0.93, respectively) reflecting a mixed diet.

Concerning Cu and Zn, their levels in bones have been recognized to be linked to a protein diet, based on the higher levels of these elements found in red meat, offal, and dairy products respect to other foods. We found significantly higher Cu (34.6 μg/g) and Cu/Ca ratio (0.14) in Sassari (Tables 2 and 3) referring to more animal foods consumption in this area. The optimal values of Cu/Ca are offered as between 0.15 and 0.20 (Giorgi et al. 2005). Considering that subjects found in Sassari were buried wearing gifts or bronze ornaments, a likely *post-mortem* contribution to the observed Cu concentration could be possible.

Significantly higher levels of Zn (350 μg/g) and Zn/Ca ratio (1.52) were observed in Bisarcio site, indicating a diet more abundant in sources as meat, dairy products, and some vegetables such as nuts and legumes (Tables 2 and 3). In addition, the significant correlation we found between Ba/Ca and Zn/Ca ($\rho = 0.591$, $p = 0.026$) in Bisarcio confirmed this kind of diet. Reference Zn levels in mammalian bones of herbivorous (90–150 μg/g) were lower than those found in bones of carnivorous (175–250 μg/g) (János et al. 2011). Moreover, the Zn/Ca value was also used for classifying the type of current economy of the investigated population; a higher ratio (> 0.60) was indicative of a rich economy, while a lower ratio (< 0.35) of a poor in protein economy (Giorgi et al. 2005).

Regarding gender variable, the Mann–Whitney test revealed no differences between males and females of the same excavation area (data not shown), indicating that there were no variations of diet and habits linked to sex. Considering age classes, we found significant differences, obtained with Kruskall–Wallis test, in bone median values of Cu ($p < 0.001$) and Cd ($p = 0.008$) which were both higher in the younger group (≤ 24 years) (Fig. 2). Also ratio levels of Cu/Ca (P50, 0.08 vs. 0.05; $p < 0.001$) and Cd/Ca (P50, 0.0013 vs. 0.0007; $p = 0.02$) were significantly higher in the ≤ 24 years group. Because we did not find lower levels of Ba, Sr, and Zn (and their ratios with Ca) in the younger population, specific conditions like insufficient nutrition or poorer living conditions in the sub-adult population respect to adults can be excluded (Stipisic et al. 2014). Higher levels of metals including Cu, Sr, and Zn in bones of subadults than adults were also found by other authors who proposed reasons as different biogenic accumulation, development of digestive tract, or susceptibility of subadult bones to diagenesis (Güner et al. 2011; Zapata et al. 2006; Lambert et al. 1984).

Considering types of bones, data reported in Fig. 3 revealed significantly higher median levels of Sr ($p < 0.001$) in fibula and Ba ($p = 0.011$) in humerus respect to the other bones. The same dissimilarity between fibula (P50, 2.61) and other bones (P50 range, 0.16–0.30) was observed for Sr/Ca ($p < 0.001$) and between humerus (P50, 0.66) and other bones (P50 range, 1.06–1.61) for Ba/Ca ($p = 0.007$). These differences were conditioned by the high number of these types of bones existing in the two sites of Geridu and Bisarcio; for this reason, the enriched Sr and Ba levels (from two to four times) observed in fibula and humerus can be ascribed mainly to the area where bones were collected.

In order to better visualize the results, bivariate plots of Ba/Ca vs. Sr/Ca and Zn/Ca vs. Sr/Ca were made (Figs. 4 and 5, respectively). We confirmed that Bisarcio and Geridu showed isolated groups of individuals; those with a terrestrial-based diet living in the first site (abundant in Ba and Zn) and those with a vegetarian and marine origin diet (abundant in Sr) living in the second area. To the opposite, the two sites of Alghero and Sassari overlapped indicating that in these locations, subjects did not consume a "peculiar" diet.

All these findings could be explained in view of the geographical and social background of the four study areas (Fig. 1). Individuals from Alghero and Sassari can be considered as living in a society with middle- to high-income economies, mainly based on intense commercial activities, food imports included. As a consequence, no clear distinction between bone levels of Ba, Cu, Sr, and Zn was observed reflecting that individuals of these two locations consumed various sorts of food, also imported foods. To the opposite, the sites of Bisarcio and Geridu were very small villages. Bisarcio was far from the sea (ca. 50 Km), and the higher levels of Ba and Zn in bones reproduced the agricultural and hunting/breeding practices preponderant in this site. The inhabitants of Geridu could easily introduce also marine food in their diet, and this could contribute (together with vegetables) to the higher Sr levels found in this location; in fact, Geridu was not so distant from the sea and, at that epoch, was largely involved in commercial trades with the more developed town of Sassari. Previous investigations showed very low mean Zn

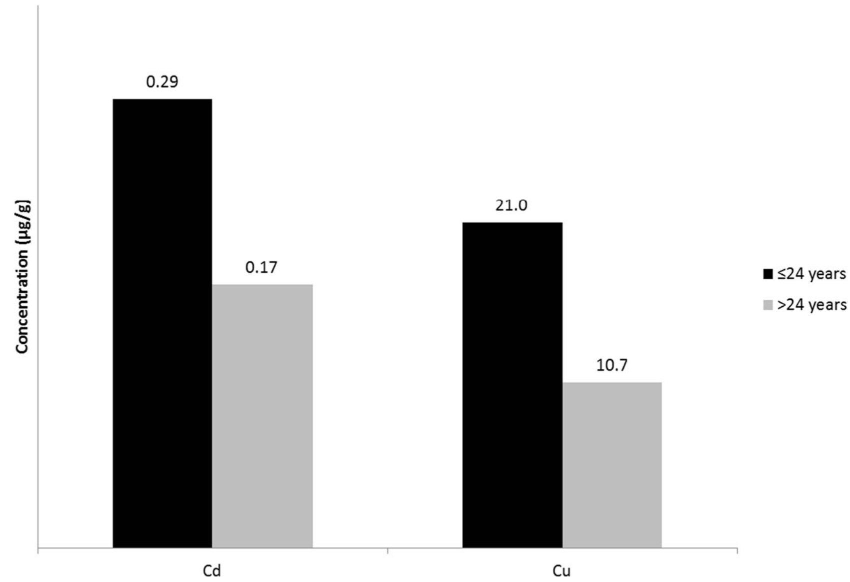

Fig. 2 Metals statistically different by age classes

concentrations (< 100 mg/kg) and elevated levels of Sr (ca. 400–500 mg/kg) in the middle-aged bones (in tenth and ninth century AC) in Hungarian and Croatian burial sites, which might probably be connected with heightened consumption of vegetables, cereals, and legumes (János et al. 2011; Stipisic et al. 2014). In an Early Medieval (sixth to eighth century AC) graveyard in Germany, the dietary reconstruction was made by the use of Ca, Sr, Ba, Cu, and Zn values; the general diet was likely to have been based on considerable amounts of vegetable food items (cereal grains and leafy vegetables as a main dietary component) for the high levels of Ba (ca. 100 mg/kg) and Sr (ca. 450 mg/kg) beside the intake of milk products and legumes for Zn (Schutkowski et al. 1999). Allmäe et al. (2012) studied different types of bones (from the seventh to the eighteenth century AC) coming from different three Estonian cities either coastal or terrestrial; data indicated a relatively mixed diet (enriched in Sr, Cu, and Zn) for the coastal village and mainly vegetarian (high in Ba) for the rural one.

As regards Cd, Hg, and Pb, they do not play any known beneficial role in the human organism and are considered toxic elements with acute and chronic effects. Regarding Cd, our median results ranged between 0.18–0.27 µg/g and pointed out no differences between the four archeological sites. For Cd, bone concentrations reported in literature spanned between 0.05 and 8 µg/g, the higher values corresponding to present-day individuals (Jaworowski et al. 1985; Güner et al. 2011; Martínez-García et al. 2005). Regarding Hg and Pb and their ratios with Ca (Tables 2 and 3), significantly higher

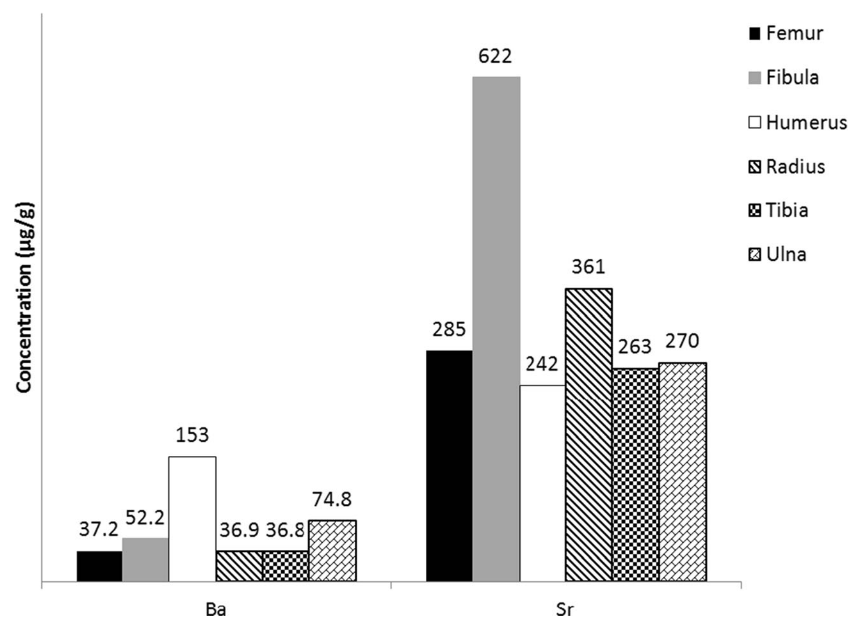

Fig. 3 Metals statistically different by type of bone

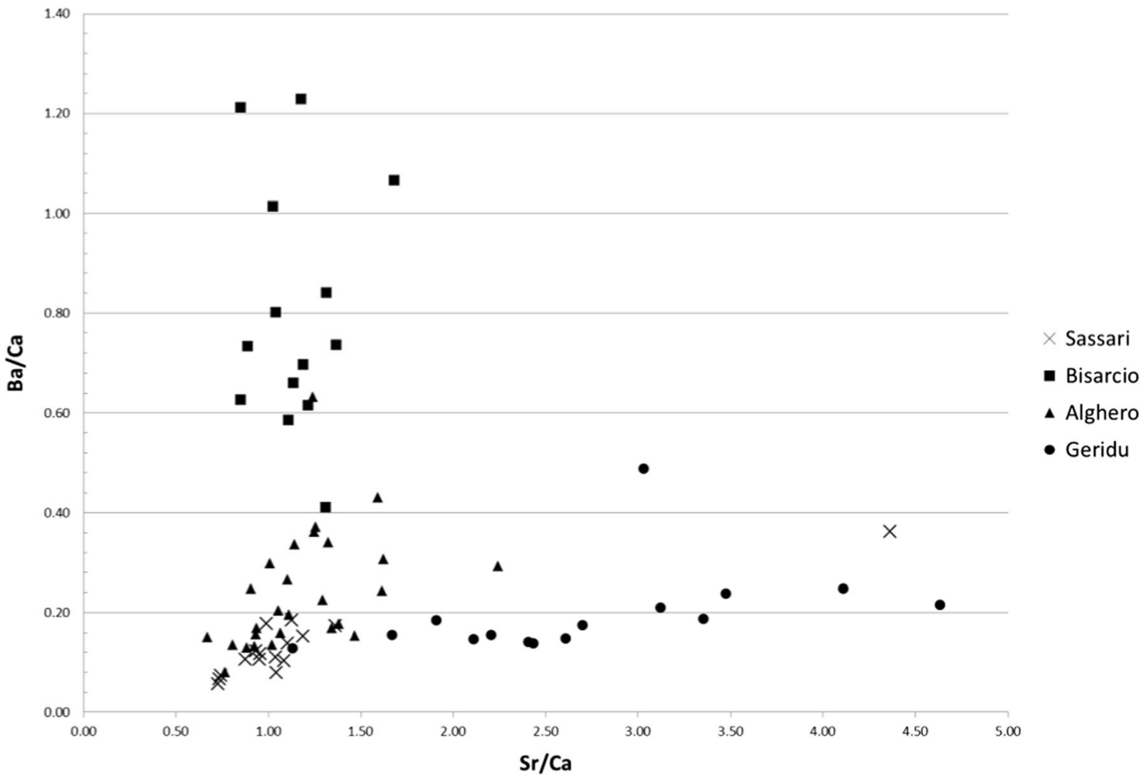

Fig. 4 Bivariate plot of Ba/Ca vs. Sr/Ca in bones collected at four archeological sites

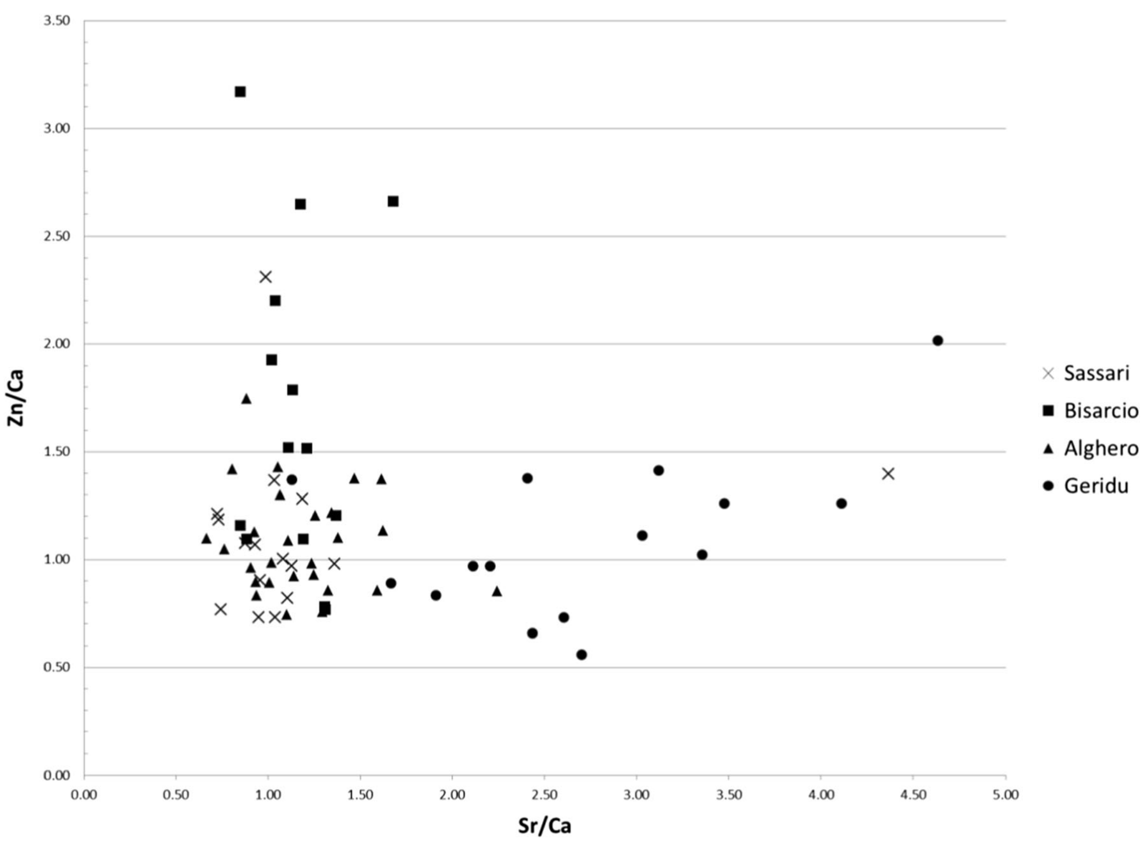

Fig. 5 Bivariate plot of Zn/Ca vs. Sr/Ca in bones collected at four archeological sites

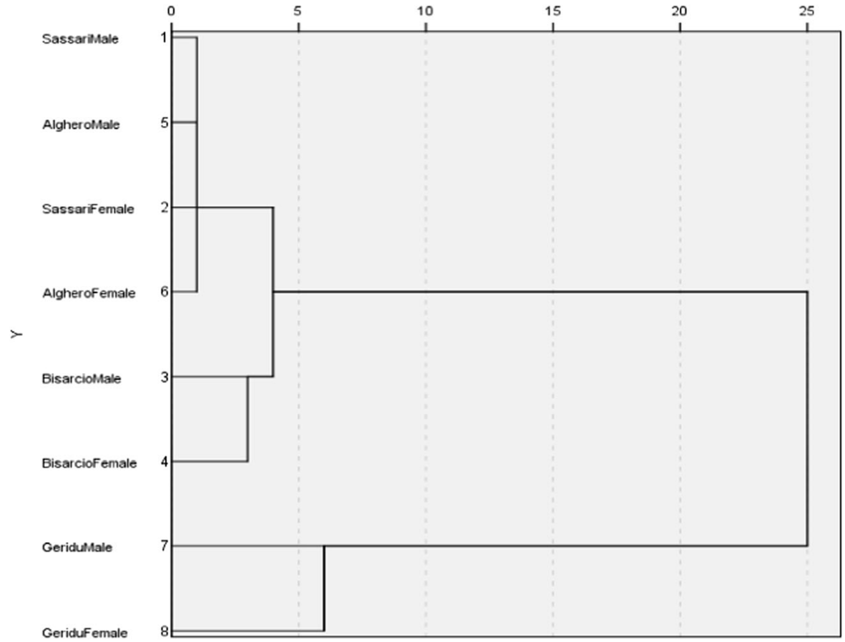

Fig. 6 Plot of CA using Ba/Ca, Cd/Ca, Cu/Ca, Hg/Ca, Pb/Ca, Sr/Ca, and Zn/Ca in bones and gender

levels were observed in individuals from Alghero and Sassari respect to people from Bisarcio and Geridu. As already stated, the former sites are characterized by a more urbanized and anthropic environment respect to the rural environment present in Bisarcio and Geridu. For this reason, we supposed that in Alghero and Sassari, some point sources and lifestyles (as drugs, pigments, paints, amalgams, cosmetics) would have contributed to the higher levels of Hg found in bones of these areas. Moreover, given the presence of the port in Alghero, another source for the higher values of bone Hg could be the consumption of fish. Mercury in our samples exhibited levels comparable with those previously reported in bones which have experienced an *intra-vitam* exposure to Hg (0.27 μg/g) (Rasmussen et al. 2013). Lead can be considered ubiquitous in the environment of medieval times, e.g., in the glazing of ceramics used for foodstuff, coins, roof tiles, window panes, and water pipes. Diverse authors have highlighted the fact that modifications induced by anthropic activity are not an exclusive product of modern times, and the discovery of mining and metal processing provided the biogenic uptake of metals via feeding, drinking, breathing, or contact (Martínez Cortizas et al. 1991; Nriagu 1996). In any case, our data on bone Pb (maximum median value of 15 μg/g) were significantly lower than those in bones from different historical epochs such as Bronze age, 578 μg/g; Roman epoch, 369 μg/g; Byzantine period, 459 μg/g; Islamic period, 316 μg/g; and present-day, 82.5 μg/g (all expressed as mean value) (Martínez-García et al. 2005), and also lower when compared to extreme cases in industrial populations of the twenty-first century (P50, 45.15 μg/g) (Baranowska et al. 1995).

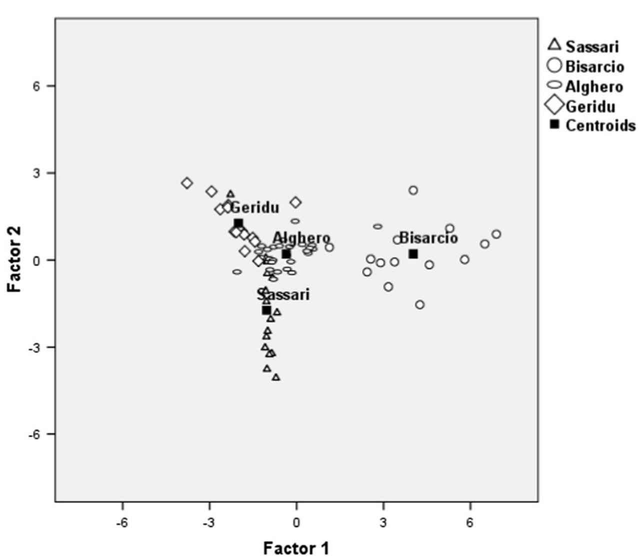

Fig. 7 Plot of LDA using Ba/Ca, Cd/Ca, Cu/Ca, Hg/Ca, Pb/Ca, Sr/Ca, and Zn/Ca in bones and archeological sites

Multivariate analysis

Multivariate analyses (CA, LDA, and PCA calculations) on Ba/Ca, Cd/Ca, Cu/Ca, Hg/Ca, Pb/Ca, Sr/Ca, and Zn/Ca ratios were performed. Results of the CA applied to the different areas (Fig. 6) indicated one cluster with a strong association between females and males subjects living in Alghero and Sassari. Individuals of Bisarcio and Geridu formed isolated clusters with a weak association with individuals from other areas. The LDA model correctly classified the 85% of samples according to the living areas (Fig. 7). In particular, the 93% of individuals were classified in Bisarcio, 80% in Geridu, and 93% in Alghero. A lower level of classification was obtained for samples collected in Sassari (69%) with the 25% of samples attributed to Alghero, testifying the similarity between the two populations. The PCA applied to metals/Ca ratios (Fig. 8a, b), using three components (> 1), indicated the 28.7% of the variation was explained by the first component (PC 1), the 24.2% by PC 2 and the 14.4% by PC 3, as shown by factor loadings of Fig. 8a. The ratios Ba/Ca (weight of 0.893) and Zn/Ca (weight of 0.870) were important for the construction of the first factor; both ratios showed high positive loadings indicating they have a similar nutritional origin. The second factor was explained by the high positive coefficient of Cu/Ca (weight of 0.835) and Pb/Ca (weight of 0.721) and the high negative coefficient of Sr/Ca (weight of − 0.637), indicating opposite origins of these metals. In the case of Cu and Pb, their common origin can be the meat-based diet as well as exposure to metallic objects daily used. The Cd/Ca (weight of 0.822) and Hg/Ca (weight of 0.752) ratios resulted relevant for the third factor, indicating that these two metals were mainly attributed to environmental exposure rather than to diet. Considering the score plots reported in Fig. 8b, individuals living in Bisarcio were enriched in Ba/Ca and Zn/Ca levels confirming they had a similar diet based on terrestrial nutrition; while subjects from Geridu were enriched in Sr/Ca levels because of their vegetarian or marine-based diet. These results confirmed those obtained by the LDA model. Moreover, although the diet of some subjects of Sassari was influenced by a high intake of Cu, this group partially overlapped that of Alghero (as showed also by the LDA model). Finally, the population of Alghero was very close to the "0-point," confirming subjects used prevalently a mixed diet.

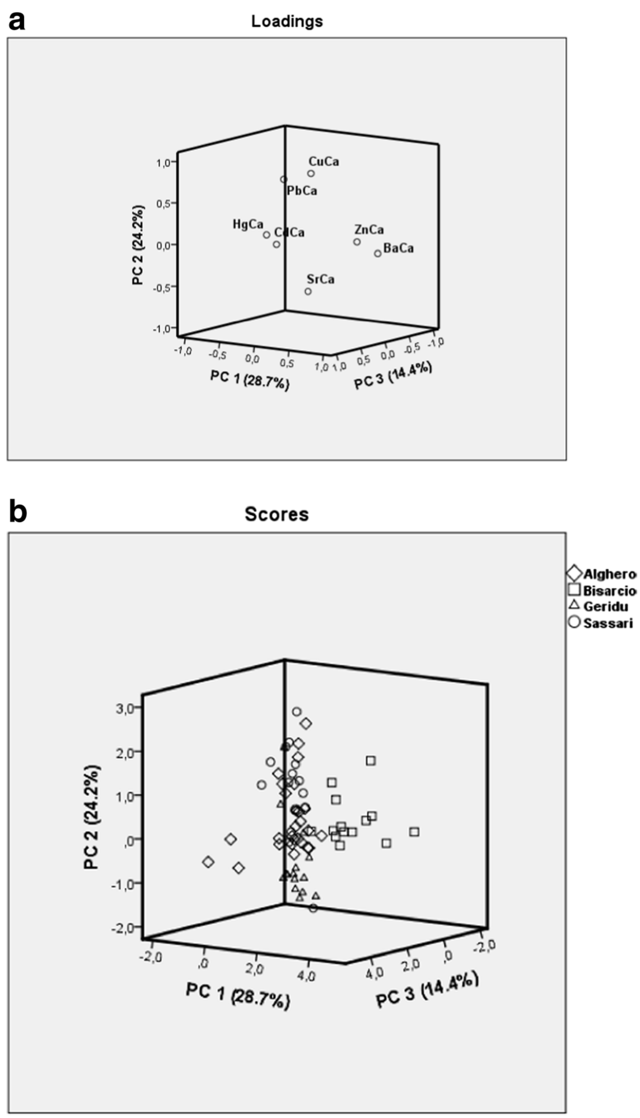

Fig. 8 **a** Factor loadings determined by PCA of Ba/Ca, Cd/Ca, Cu/Ca, Hg/Ca, Pb/Ca, Sr/Ca, and Zn/Ca in bones collected at four archeological sites. **b** Scores determined by PCA of Ba/Ca, Cd/Ca, Cu/Ca, Hg/Ca, Pb/Ca, Sr/Ca, and Zn/Ca in bones collected at four archeological sites

Conclusion

Elemental quantification in pre-historic mineralized human bones is important to correlate dietary habits and environmental conditions of the Sardinian population sub-grouped by archeological sites. A general result was that the manner of incorporation differs depending on the metal; some metals (Ba, Sr, Cu, and Zn) are incorporated predominantly by diet and others are introduced by the environment or lifestyle (Cd, Hg, and Pb). In particular, the higher bone Sr and Sr/Ca levels in Geridu were indicative of plant foods consumed in this location, integrated by marine products indicating also fishing activities. The higher bone Ba and Ba/Ca levels in Bisarcio showed a diet enriched in animal proteins, reflecting agriculture and cattle breeding as the main activities. No difference was observed in Sr and Ba content and their ratios with Ca in Sassari and Alghero; this was ascribed to a more mixed diet consumed in these sites where a higher developed economy with food imports was present. In addition, the higher socio-economic status of Sassari and Alghero also led to an increase in bones of metals as Hg and Pb; this suggested that already in the middle age, the exposure to toxic metals in urbanized sites respect to rural villages could be significant.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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