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Heavy metals in human bones in different historical epochs

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Abstract

The concentration of the metals lead, copper, zinc, cadmium and iron was determined in bone remains belonging to 30 individuals buried in the Region of Cartagena dating from different historical periods and in eight persons who had died in recent times. The metals content with respect to lead, cadmium and copper was determined either by anodic stripping voltammetry or by atomic absorption spectroscopy on the basis of the concentrations present in the bone remains. In all cases, zinc and iron were quantified by means of atomic absorption spectroscopy. The lead concentrations found in the bone remains in our city are greater than those reported in the literature for other locations. This led to the consideration of the sources of these metals in our area, both the contribution from atmospheric aerosols as well as that from the soil in the area. Correlation analysis leads us to consider the presence of the studied metals in the analysed bone samples to be the consequence of analogous inputs, namely the inhalation of atmospheric aerosols and diverse contributions in the diet. The lowest values found in the studied bone remains correspond to the Neolithic period, with similar contents to present-day samples with respect to lead, copper, cadmium and iron. As regards the evolution over time of the concentrations of the metals under study, a clear increase in these is observed between the Neolithic period and the grouping made up of the Bronze Age, Roman domination and the Byzantine period. The trend lines used to classify the samples into 7 periods show that the maximum values of lead correspond to the Roman and Byzantine periods. For copper, this peak is found in the Byzantine Period and for iron, in the Islamic Period. Zinc shows an increasing tendency over the periods under study and cadmium is the only metal whose trend lines shows a decreasing slope.
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1. Introduction

Cartagena was in antiquity one of the most important and prosperous cities on the Iberian

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Peninsula, a fact demonstrated both by ancient written sources as well as by archaeological studies.

Several factors converge in the causes that brought about its growth, some rooted in politics and economy and others of a geostrategic character. Its privileged position, on what in the past was a peninsula surrounded by hills overlooking the sea, closed off by a lagoon that spread out behind it, made the city and its bay one of the best protected natural enclaves in the Mediterranean.

The exceptional conditions of its port soon facilitated intense commercial activity, fomented by its proximity to the north of Africa, by its easy accessibility with respect to the interior of the Iberian Peninsula and above all by the enormous riches of its mines.

Although it was Carthage's interests in establishing itself in this territory that resulted in making it the capital of the Barca dynasty in Iberia in the year 229 B.C., the fact is that the mining tradition of this region had already boasted a well-earned reputation with respect to metals since the first millennium before Christ, especially lead and silver, which were elaborated in numerous factories that stood along the mountainous shoreline, from Mazarrón to Cabo de Palos.

Even though Prehistoric ore mining is documented among the indigenous populations of the region, early production of copper hardly reached the threshold of self-sufficiency, contrary to what occurred in other areas of the eastern Mediterranean. The so-called Chalcolithic Period, implanted here from the third millennium B.C., barely incorporated objects made from this metal, making them an almost exotic element solely possessed by the most influential local castes.

It was to be from the 2nd millennium B.C., at the height of the Bronze Age, when objects made from this metal reflect the full adoption of the working of this metal by the peoples of the Argaric Culture, giving rise to widespread production organised in specialised guilds and workshops. At the end of this period, and above all in the early centuries of the 1st millennium B.C., we already find small factories producing lead and silver, now linked to the true mining potential of the subsoil of the region of Cartagena. The sites of these small foundries, many of which were located just a few metres from the coast, was to largely obey the growing demand for these products and the strong trading links that by then existed between local mining and the Phoenician world and its intermediaries, who controlled sea traffic on this side of the Mediterranean.

One of the most recent archaeological findings, that of two ships dating from this period sunk in the Port of Mazarrón (Barba et al., 1995), highlights up to what point local mining was the object of trade. The majority of its heavy cargo, undoubtedly taken on board in this port of call, was made up of ingots of litharge, the goods from the point of origin being ceramic dishes, so appreciated by the local peoples.

A few hundred metres from the site of this finding, on the small tombolo at Punta de los Gavilanes, (Ros et al., 2003), a small factory has been documented that possessed foundry ovens used to obtain this product, thus confirming the clearly commercial vocation of these strategic coastal installations.

The dynamics of exchange grew at the same rate as cupellation techniques evolved. The increasing demand for lead in the centuries to come made production grow along with the number of establishments and the size of the foundries treating silver-bearing galenite. The example of Los Nietos, an Iberian settlement from this period on the shore of the Mar Menor (Garcia, 2001), clearly illustrates how in the 4th century B.C. its inhabitants had achieved a certain degree of mining specialization, by now a long way from the traditional agricultural and cattle farming activity of their contemporaries in inland rural settings.

The diversification of tasks related to mining was by now a fact, as the very existence of this settlement proves. The work of extracting the ore in the neighbouring range of La Unión contributed the raw material that had to be smelted in the ovens at this location, frequented by traders in charge of carrying out transactions.

It is not surprising then that Carthage set its eye on this coastal strip when establishing its main base on the Peninsula, thus counteracting the losses it had incurred after the first Punic War. This expansive policy of the Barca family was to be culminated by Hasdrubal's founding of a new city, Qart-Hadash, called to become the capital of the Punic territories in Iberia.

But the Carthaginian project was soon to be aborted, and with it, the control of the mines. Conscious of the economic interest of these metals, after the conquest of the city, Rome established an ambitious exploitation project, the control of which was initially assigned to the state magistrates. During this period, installations spread all over the mountain chain multiplied and their products were shipped from small quays to Carthago Nova, this port being a hub for their large-scale commercialisation.

The strict state control established after the early years of the conquest was soon to be ceded to societates publicanorum, subsequently passing into private hands of Italic origin. The study of the cartouches of the numerous ingots found to date enables us to ascertain the existence of more than 10 families who controlled the exploitation of the mines in the environs of Carthago Nova, among these the Aquini, Messi, Planii or Atelli, the same as those who occupied the main public posts in the city.

For reasons still not fully understood, mining activity in Carthago Nova underwent a serious regression at the end of the 1st century A.D., from which it was not to recover, at least with the same intensity it had maintained between the 2nd century B.C. and the 1st century A.D.. Said period was one of feverish activity, which ancient sources describe with singular realism. Among these, the historian Polybius, who when mentioning the silver mines, states that they are very large, that they are some 20 stadia from the city (3700 m) and are to be found within an area of 400 stadia in circumference (74 km): whose perimeter and distance from Cartagena coincide with that of the present-day mines, in which 40,000 men work constantly (Torres, 1982). Polybious narrates, in reference to Estrabón concerning mining in the Sierra de Cartagena: "that the exploitation of silver was done in a particular way, and that on certain days the State perceived for the tax that it imposed (which summed a quarter of the product) profits of up to 15 to 20,000 drachma" (from 58,500 to 78,000 reales) (Estrabón, Book III). These figures refer to the years 140 to 130 B.C., the dates when Polybius made his journeys, visiting Cartagena. Estrabón called Carthago Nova "extremely opulent due to its silver mines". The Romans also developed an impressive lead technology, transporting water via piping made from this metal, using it in kitchen utensils, adding it to wine, etc. (Boeckx, 1986). At the height of the Roman Empire, lead production was around 80,000 tons per year (Settle and Patterson, 1980).

When the Visigoths arrived, they found a moribund mining industry as a consequence of the profound decadence in which mining work had fallen in the last years of Roman domination, although in the Visigoth period, the Roman organisation and legislation of mining still continued and the mining systems were maintained. The mining waste from the Roman period was exploited to extract lead and silver (Molina López, 1986). During this period, metals were worked by Spanish craftsmen and there are reliable reports of an extraordinary flourishing of the working of precious metals, of the weapons industry and of other metallic arts.

The most diverse authors coincide in the conviction that the Muslims continued the designs of the Visigoth period in mining. Referring to mining up until the 11th century, Sánchez Albornoz writes, "we have reports of the practice of extractive industries. We are unaware of what the exploitation system of the mines would be, but it is known that no mining royalty existed as in the Empire" (Peñarroya-España, 1881).

In the 13th–14th centuries, however, a collapse occurred in mining in the Sierra de Cartagena as a result of the wars and plague epidemics that decimated the population and in the 15th century there was a decline attributed to the effects of the economic crisis and the mining boom in the New World. Licenciado (Graduate) Cascales (1874) points out that in the year 1614 "The city of Cartagena, which was at one time the India of the Romans, today has hills from whose soil silver is extracted, as I have seen smelted in an assay that by order of Don Felipe II was carried out to evaluate the cost along with the interest". The stagnation of the mines must have been especially serious in the area of Cartagena in the 18th century, the mines being shut down, the miners not considering their exploitation profitable at that time.

In the 19th century, from the Law of 1825 on, there is the beginning of a resurgence in the mining industry in Cartagena and a recovery of lead mining. As an example of the boom of 1851, there were 290 working concessions and 45 lead smelting factories functioning, obtaining 15,016 tons of lead from 207,000 tons of ore. In 1862 the figures were even greater: 17,384 and 175,000 tons, respectively, the consequence of mechanisation. The local mining district dominated the lead mining sector between 1861 and 1900, producing 56% of the ore and 24.2% of the metal. From 1860, zinc ore begun to be extracted, which came to represent 21.6% of the national average in that period (Egea Bruno, 1996). From 1868, the proliferation of mining activities led to a rapid growth in population and the creation of several miners' settlements. Iron began to be mined at the end of 1860 and in the 1980s, annual production was established around 600,000 tons (De-Botella and De-Hornos, 1868).

There exist numerous veins in the Sierra de Cartagena, although these do not usually reach the surface, particularly in the space that comprises certain eruptive masses. These veins are almost always covered by thick ferruginous masses heavily loaded with lead, which under the name of carbonates form the object of the main mining activity (De-Botella y De-Hornos, 1868).

We therefore find ourselves in a territory where the soils are rich in metals, areas existing where mining activity has taken place. This activity undoubtedly meant that the inhabitants of each epoch were in contact with some metals that might have entered into their organism on account of their bioavailability. The way these metals entered the organism might have been via ingestion, via polluted water or foodstuffs or via inhalation and cutaneous adsorption. Lead accumulates mainly in the bones (Rabinowitz, 1991) and teeth (Fergusson and Purchase, 1987) in the form of chelates (Vega, 1988), 95% of total body lead being found in the bones. Lead has toxic effects on many organs, systems and physiological processes, above all on the development of the central nervous system (Sanín et al., 1998). The neurotoxicity of lead is more critical for the developing foetus and the growing child. The toxic cations of lead are attributed to its affinity for the molecular action sites of calcium, acting as its substitute in intracellular processes.

The aim of the present study is to obtain an approximation of the values of certain metals in the bones of inhabitants from different epochs using the human remains that have appeared in archaeological excavations and comparing them with the values found in individuals who have died in recent times.

2. Materials and method

The study was carried out on human bones that had been found at different archaeological excavations and that were deposited in the Municipal Archaeological Museum, and which belonged to 30 individuals from different epochs. With the aim of completing the study, we used 8 samples ceded by the Anatomical Forensic Institute corresponding to present-day bones.

The bones were supplied to us already classified into 6 historical periods on the basis of dating carried out by specialist in this field, plus the group of present-day remains, thus resulting in 7 groups that were assigned the following codes: Neolithic, Epoch 1 (n=4); Bronze Age, Epoch 2.1 (n=2); Roman, Epoch 2.2 (n=11); Byzantine, Epoch 2.3 (n=12); Islamic, Epoch 3.1 (n=37); 18th century, Epoch 3.2 (n=6) and present-day remains, Epoch 4 (n=8). Given the small sample size of some of these groups, in order to be able to analyse the differences existing between these from a statistical estimation, we grouped together the results corresponding to the different historical epochs, obtaining an initial classification in four groups, numbered 1 to 4. The first, Epoch 1 (n=4), continues to correspond to the Neolithic period, the second, Epoch 2 (n=25) includes the remains from the Bronze Age, the Roman Epoch and the Byzantine Period, the third, Epoch 3 (n=43) comprises the results of the remains of the Islamic period and the 18th century, and the fourth, Epoch 4 (n=8) corresponds to present-day bone remains. Finally, all the results of the remains from the Bronze Age until the 18th century were grouped together into one single group, thus obtaining three categories, A, B and C: prior to the age of metals (A, n=4), from the Bronze Age till the 18th century (B, n=68) and present day (C, n=8).

In order to assess soil moisture contamination, Barium and Calcium were determined in the bones and the ratios Ba/Ca and Pb/Ca were calculated following Ericson et al. (1979) and Ericson (1982).

The metals content was determined in all the remains: lead, cadmium, copper, iron and zinc. To do so, the samples were duly prepared and the necessary analyses carried out, using the certified bone standard SRM 1486 as reference material.

The different human bone remains were processed free-of-soil (those belonging to the Municipal Archaeological Museum, with their registration number and piece labelled with the name of the site at which they were found). All the bones were subjected to calcination at 500 °C for 2 h so as to eliminate organic matter.

Analytical determination was carried out using anodic stripping voltammetry (ASV) and flame atomic absorption techniques. To do so, we used the Metrohm 626 polarograph equipped with stand 663 and controller 608 and the Perkin Elmer 3100 double beam spectrophotometer, equipped with a deuterium background corrector and high sensitivity nebuliser, always carrying out weighings of less than 0.5 g (Mettler AJ100 analytical balance). Tests were carried out using acid digestions either in a closed pressurised atmosphere in Teflon flasks in a microwave oven (CEM Mars 5), or in an open atmosphere with different acid mixtures, until reaching equilibrium between the consumption of reagents and the goodness of the digestion. The chosen acid mixture was 7 ml of nitric acid and 2 ml of perchloric acid, both of Suprapur® quality (Ref. 100517, 100441). Once the acid mixture had been added, the sample beaker was then placed on a heating plate until dryness. The precipitate was redissolved in double distilled water and placed in a 25-ml volumetric flask and levelled off. One out of each ten samples was from the SRM 1486 bone standard, the composition of which is certified.

To determine lead, cadmium and copper by anodic stripping voltammetry, 2 ml aliquots from the 25 ml flask were placed in the polarographic vessel with a background solution (perchloric acid and water). Quantification was carried out using the addition method, preparing a standard of three metals with double distilled water containing 8 ng of cadmium, 80 ng of lead and 80 ng of copper, from standard TITRISOL® solutions from Merck (Ref. 109960, 109969, 109987).

The elements whose concentration exceeded the sensitivity limit of the above technique (in all cases for zinc and iron, and depending on the sample of lead and copper) were analysed using the flame atomic absorption technique employing standard solutions from Merck (Titrisol® Ref. 109953, 109972), directly measuring in the 25-ml volumetric flask against standard solutions of the corresponding metals: lead, copper, iron and zinc.

The data from the samples and the results of the analyses were used to construct a database in Excel that was exported for statistical treatment of the data using the SPSS 11.0 software package.

3. Results

Skeletal ratios of Barium to Calcium do not vary much within humans for different geographic areas and chronologic ages (Ericson et al., 1979). A factor of 10 for change in Ba/Ca ratio (one logarithmic unit) is the maximum variation possible due to biopurification (Burton et al., 2003). As the values of the Ba/Ca ratio of the analysed archaeological remains were found to be below that of the bone remains of an individual from the Anatomical Forensic Institute, who had not been buried, and since the range of values found for each period studied for this ratio does not exceed the maximum attributable to biopurification (Burton et al., 2003), indicating that the differences may be due to variations in diet or to excessive metabolic uptake (Manea-Krichten et al., 1991), we did not eliminate any of the samples from the subsequent data analysis.

Table 1 shows the complete set of bones employed in the study, with the individuals from which samples were obtained, the anatomic location, the epoch in which they were dated by specialists in the matter, the estimated age according to archaeologists and the burial site, the type of bone analysed, along with the analytical results for the metals studied in each of the samples. Of the 38 individuals from whom we were supplied bones, we obtained 80 samples, 72 from historical periods, 4 prior to the age of metals (Neolithic Epoch) and 68 from the Bronze Age until the 18th century, and 8 from individuals who had died in the 21st century, which served as a contrast.

Table 1
Concentrations in ppm of the metals lead, copper, zinc, cadmium and iron in human bones

Individual	Age	Epoch	Site	Piece	Pb ppm	Cu ppm	Zn ppm	Cd ppm	Fe ppm
1	A	3.2	Mayor Street	radius	29.8	16.6	147.5	0.89	213.6
				femur	8.9	7.4	133.4	0.49	36.2
				tooth	11.7	3.9	186.5	0.21	399.4
2	A	2.2	Old Cathedral	rib	683.0	276.8	442.0	20.1	481.0
				humerus	79.8	203.2	151.2	9.43	123.4
				tooth	32.7	11.4	168.0	0.66	18.9
3	A	2.2	Torreciega	femur	80.0	13.36	122.1	1.11	2545.2
				theet	193.2	13.3	248.4	0.67	54.0
4	A	3.2	Milagrosa niches	humerus	253.7	6.55	201.0	3.12	4974.0
5	A	3.2	Amphitheatre	radius	167.5	6.0	215.2	1.14	1096.7
				tooth	23.7	0.69	123.38	0.0062	62.6
6	A	3.1	Cuatro Santos Street	tibia	167.7	3.12	114.75	1.57	143.5
				tooth	44.4	3.13	172.2	0.62	45.71
7	A	3.1	Cuatro Santos Street	Ulna	299.7	11.57	152.62	3.84	588.2
				femur	82.5	6.62	130.86	1.56	337.1
				tooth	12.8	2.38	101.0	0.0055	42.0
8	A	3.1	Cuatro Santos Street	femur	615.4	55.0	214.0	19.0	6039.5
				radius	665.0	91.3	376.0	0.58	8123.5
				femur	437.0	42.5	151.0	0.28	1750.0
9	A	2.3	Marango Street	tibia	590.0	80.5	212.0	0.5	1858.0
				femur	902.5	118.0	532.0	0.61	9319.5
10	A	2.3	Marango Street	tooth	108	28.0	156.0	0.19	1620.0
				femur	13.25	20.2	154.0	0.38	81.6
11	A	2.3	Marango Street	femur	67.5	17.6	110.2	0.44	325.6
				humerus	42.4	16.0	101.5	0.44	214.0
12	A	2.2	Villa del Rihuete (Mazarrón)	rib	1035.0	42.6	213.0	0.37	2028.0
				tooth	71.5	36.8	89.0	0.033	88.0
				radius	798.0	47.6	224.2	0.29	1619.0
13	A	2.2	Villa del Rihuete (Mazarrón)	rib	393.0	69.0	233.3	0.65	983.0
				mandible	596.0	46.0	116.8	0.34	118.0
				tooth	103.3	113.2	165.0	0.123	231.6
14	A	2.1	Punta de Gavilanes Mazarrón	tarsus	924.6	53.6	137.3	7.69	398.0
				fibula	232.7	40.0	139.6	7.0	221.0
15	A	1	Las Amoladeras	fibula	45.0	39.0	164.0	0.61	487.0
				radius	20.2	7.0	132.3	0.19	191.5
				rib	19.8	6.3	128.7	0.045	159.2
				tooth	6.9	1.66	99.0	0.006	31.2
16	A	3.1	Puerta de la Villa	radius	131.6	8.7	126.2	6.07	1824.0
				humerus	282.6	58.25	214.1	0.62	8105.0
				rib	277.7	52.0	271.7	14.4	7920.0
				tooth	107.0	1.09	83.8	0.031	98.0
				tooth	97.1	9.76	104.4	0.084	241.5
17	A	3.1	Puerta de la Villa	tibia	150.0	20.4	113.0	0.13	167.7
				rib	225.2	37.2	173.0	0.92	1236.0
				tooth	51.4	5.18	136.8	0.029	102.6
18	A	3.1	Puerta de la Villa	rib	389.9	45.5	255.6	1.15	2600.0
				tooth	43.0	3.8	114.5	0.024	200.3
19	A	3.1	Puerta de la Villa	rib	209.2	43.9	339.2	1.1	1555
				tooth	78.7	3.27	133	0.006	180.5
20	A	3.1	Puerta de la Villa	radius	140.3	22.0	121.8	0.053	685.8
				humerus	156.8	3.15	132.24	0.039	944.3
				femur	203.5	23.1	107.2	0.025	1502.0

Table 1 (*continued*)

Individual	Age	Epoch	Site	Piece	Pb ppm	Cu ppm	Zn ppm	Cd ppm	Fe ppm
21	A	2.3	Marango Street	fibula	742.3	111.2	306.3	1.39	3341.3
				tibia	556.4	89.3	372.4	0.79	3720.4
22	A	2.3	Marango Street	tooth	375.8	47.5	130.0	1.5	1881.5
23	A	2.3	Marango Street	vertebrae	230	75	355.2	2.8	5886.0
24	Ch	2.3	Marango Street	rib	890.0	87.4	484.0	14.2	8740.6
				vertebrae	994.7	97.1	647.4	1.7	20,956.4
25	Ch	3.1	Cuatro Santos Street	cranium	274.8	46.8	106.8	0.59	3050.4
				tooth	161.8	25.42	96.7	0.27	139.0
26	F	3.1	Cuatro Santos Street	humerus	284.0	79.0	195.0	0.39	1982.0
				rib	485.0	90.4	309.2	1.57	2664.5
27	Ch	3.1	Cuatro Santos Street	humerus	1139.0	94.2	242.5	0.34	15,383.2
				rib	898.0	65.0	215.0	0.49	816.0
28	Ch	3.1	Cuatro Santos Street	tooth	314.5	31.0	131.6	0.067	255.8
				rib	783.0	62.1	192.0	1.36	1034.0
29	Ch	3.1	Cuatro Santos Street	femur	269.0	36.2	142.0	0.27	606.0
				vertebrae	677.0	61.5	226.8	0.37	1959.5
				rib	361.0	54.0	147.8	0.418	328.0
30	Ch	3.1	Cuatro Santos Street	rib	568.0	53.3	175.5	0.25	632.0
				tooth	170.4	30.0	122.0	0.06	250.0
				cranium	455.5	47.0	98.64	0.103	1517.3
31	A	4	A.F.I.	sternum	36.5	43.0	413.5	0.25	498.0
32	A	4	A.F.I.	sternum	30.0	18.6	280.0	0.12	818.0
33	A	4	A.F.I.	sternum	34.0	23.0	264.0	0.1	776.0
34	A	4	A.F.I.	sternum	39.0	22.7	279.5	0.05	1035.5
35	A	4	A.F.I.	sternum	22.5	21.0	338.5	0.047	846.5
36	A	4	A.F.I.	sternum	39.0	25.0	233.3	0.046	1537.2
37	A	4	A.F.I.	sternum	23.5	19.0	205.5	0.049	881.0
38	A	4	A.F.I.	sternum	56.0	16.0	208.8	0.085	439.6

Individuals studied, age (A, adult; Ch, children; F, foetus), historical epoch, archaeological site and pieces analysed.

The data was initially explored focussing on the characterisation of the type of distribution constituted by each of the elements under study. None of the distributions of metals, lead, copper, zinc, cadmium and iron, contained in the bones fitted a Normal distribution (Kolmogorov–Smirnov and Shapiro–Wilk's tests, p=0.000 in all cases). The above tests were then carried out using transformed logarithmic data, fitting the distributions for lead, cadmium and iron to a log-normal distribution. The distributions of Ba/Ca and Pb/Ca fitted a lognormal distribution.

Mean values of Log Ba/Ca vary between −2.77 and −3.47 for Epoch 2, between −2.81 and −3.72 for Epoch 3 and between −2.67 and −3.63 for Epoch 4. The lowest value −2.67 (the highest value of Ba/Ca 2.1 10^{-3}) corresponds to the bone sample of a contemporary individual, provided by the Anatomical Forensic Institute.

As regards the epoch, three classifications were carried out: in seven groups: 1; 2.1; 2.2; 2.3; 3.1; 3.2; 4. In four groups: 1; 2; 3; 4 and in three categories: A, B and C.

Table 2 shows the statistics, arithmetical mean, standard deviation, and minimum and maximum values of the metals under study for each of the categories in which they were classified. When fitting the distributions of the metals lead, cadmium and iron to the log-normal distribution, the geometrical mean was calculated for these as the measure of central tendency and the geometrical standard deviation as the measure of spread (Cooper, 1993), Table 3.

Table 4 presents the correlations between the metals for the complete set of analysed samples, from all the periods, and Fig. 1 shows the graphic matrix of concentration data dispersion for the complete set of metals two at a time. Table 5 presents the bivariate correlations found for the 68 samples corresponding to Epoch B, and Fig. 2 shows the graphic matrix of metals concentration

Table 2
Statistics in ppm of the values of metals in bones classified by epochs and for the complete set under study

Epoch	*N*	Metal	Mean ppm	Standard deviation	Minimum ppm	Maximum ppm
A/1	4	Lead	22.98	15.93	6.90	45.00
		Copper	13.49	17.17	1.66	39.00
		Zinc	131.0	26.58	99.00	164.00
		Cadmium	0.21	0.28	0.01	0.61
		Iron	217.23	192.71	31.20	487.00
B	68	Lead	337.36	303.83	8.90	1139.00
		Copper	45.96	46.99	0.69	276.80
		Zinc	194.98	110.27	83.80	647.40
		Cadmium	2.03	4.21	0.01	20.10
		Iron	2186.57	3676.74	18.90	20,956.40
2	25	Lead	429.43	357.48	13.25	1035.00
		Copper	70.19	62.01	11.40	276.80
		Zinc	240.44	170.34	89.00	647.40
		Cadmium	2.94	5.03	0.03	20.10
		Iron	2674.16	4599.73	18.90	20,956.40
2.1	2	Lead	578.65	489.25	232.70	924.60
		Copper	46.80	9.62	40.00	53.60
		Zinc	138.45	1.63	137.30	139.60
		Cadmium	7.35	0.49	7.00	7.69
		Iron	309.50	125.16	221.00	398.00
2.2	11	Lead	369.59	353.35	32.70	1035.00
		Copper	79.39	86.14	11.40	276.80
		Zinc	197.55	96.25	89.00	442.00
		Cadmium	3.07	6.26	0.03	20.10
		Iron	753.65	908.61	18.90	2545.20
2.3	12	Lead	459.40	367.35	13.25	994.70
		Copper	65.65	37.82	16.00	118.00
		Zinc	296.75	183.57	101.50	647.40
		Cadmium	2.08	3.89	0.19	14.20
		Iron	4828.74	5969.98	81.60	20,956.40
3	43	Lead	283.84	257.41	8.90	1139.00
		Copper	31.86	27.79	0.69	94.20
		Zinc	168.55	67.35	83.80	376.00
		Cadmium	1.50	3.62	0.01	19.00
		Iron	1903.08	3041.60	36.20	15,383.20
3.1	37	Lead	316.47	260.72	12.80	1139.00
		Copper	35.91	27.85	1.09	94.20
		Zinc	168.66	71.35	83.80	376.00
		Cadmium	1.59	3.88	0.01	19.00
		Iron	2028.38	3188.11	42.00	15,383.20
3.2	6	Lead	82.55	103.15	8.90	253.70
		Copper	6.86	5.34	0.69	16.60
		Zinc	167.83	38.12	123.38	215.20
		Cadmium	0.98	1.13	0.01	3.12
		Iron	1130.42	1922.85	36.20	4974.00
C/4	8	Lead	35.06	10.62	22.50	56.00
		Copper	23.54	8.37	16.00	43.00
		Zinc	277.89	69.95	205.50	413.50
		Cadmium	0.09	.069	0.05	0.25
		Iron	853.98	339.40	439.60	1537.20
All	80	Lead	291.41	300.72	6.90	1139.00
		Copper	42.09	44.49	0.69	276.80
		Zinc	200.07	107.93	83.80	647.40

Table 2 (*continued*)

Epoch	N	Metal	Mean ppm	Standard deviation	Minimum ppm	Maximum ppm
All	80	Cadmium	1.74	3.94	0.01	20.10
		Iron	1954.84	3434.88	18.90	20,956.40

Epochs A or 1; prior to the Bronze Age. B; from the Bronze Age to 13th century. C or 4; died in the 21st century. 2; Bronze Age to 8th century A.D.. 3; from 9th to 13th century A.D.. 2.1; Bronze Age. 2.2; Roman Epoch. 2.3; Byzantine Period. 3.1; Islamic Period. and 3.2; Modern Epoch. N: sample size.

data dispersion corresponding to the bones from historical periods, two at a time. In both Figs. 1 and 2, the data for cadmium correspond to their transformed logarithm.

Table 3
Geometrical mean and geometrical standard deviation for the values of lead

Epoch	N	Metal	Geometrical mean, ppm	Geometrical standard deviation
A1	4	Lead	18.77	2.16
		Cadmium	0.08	7.33
		Iron	146.71	3.13
B	68	Lead	196.17	3.37
		Cadmium	0.46	6.53
		Iron	691.03	5.21
2	25	Lead	245.84	3.54
		Cadmium	0.94	4.65
		Iron	732.94	6.23
2.1	2	Lead	463.85	2.65
		Cadmium	7.33	1.07
		Iron	296.58	1.52
2.2	11	Lead	212.24	3.29
		Cadmium	0.65	6.03
		Iron	289.49	5.09
2.3	12	Lead	253.04	4.13
		Cadmium	0.94	3.14
		Iron	1996.96	5.28
3	43	Lead	172.04	3.25
		Cadmium	0.31	7.01
		Iron	667.77	4.76
3.1	37	Lead	219.56	2.61
		Cadmium	0.30	6.95
		Iron	752.74	4.51
3.2	6	Lead	38.24	3.99
		Cadmium	0.36	8.82
		Iron	319.04	6.21
C4	8	Lead	33.73	1.34
		Cadmium	0.08	1.83
		Iron	798.31	1.48
All	80	Lead	146.29	3.80
		Cadmium	0.35	6.64
		Iron	648.80	4.85

Cadmium and iron (log-normal distributions) for the different classifications of epochs. N: sample size.

The variance homogeneity test was carried out with the aim of studying the differences existing in the values of the metals grouped according to different epochs, using the classification in Epochs 1 to 4 as the factor, finding equal variances only for the transformed logarithmic cadmium variable. We hence rejected the use of analysis of variance to define the existence of differences attributable to this factor for these metals, carrying out instead the non-parametric Kruskal–Wallis test for these metals. Table 6 shows the level of significance for the Kruskal–Wallis test carried out for lead, copper, zinc and iron and of the ANOVA carried out for cadmium. Table 7 shows the results of the comparison of means test for independent samples carried out for all the metals.

Figs. 3–7 present the mean for each of the metals with a confidence interval of 95% for each of the four periods mentioned above. The mean values given for cadmium and their confidence level

Table 4
Correlations between the metals in bones for the complete set of 80 analysed samples

		Lead	Copper	Zinc	Cadmium	Iron
Lead	Pearson's Coeff.	1	0.553**	0.489**	0.280*	0.583**
	Bilat. Sig.		0.000	0.000	0.012	0.000
Copper	Pearson's Coeff.		1	0.541**	0.517**	0.349**
	Bilat. Sig.			0.000	0.000	0.002
Zinc	Pearson's Coeff.			1	0.286*	0.660**
	Bilat. Sig.				0.010	0.000
Cadmium	Pearson's Coeff.				1	0.218
	Bilat. Sig.					0.052
Log Cd	Pearson's Coeff.	0.429**	0.492**	0.365**	0.681**	0.328**
	Bilat. Sig.	0.000	0.000	0.001	0.000	0.003

* The correlation is significant at the 0.05 level (bilateral).
** The correlation is significant at the 0.01 level (bilateral).

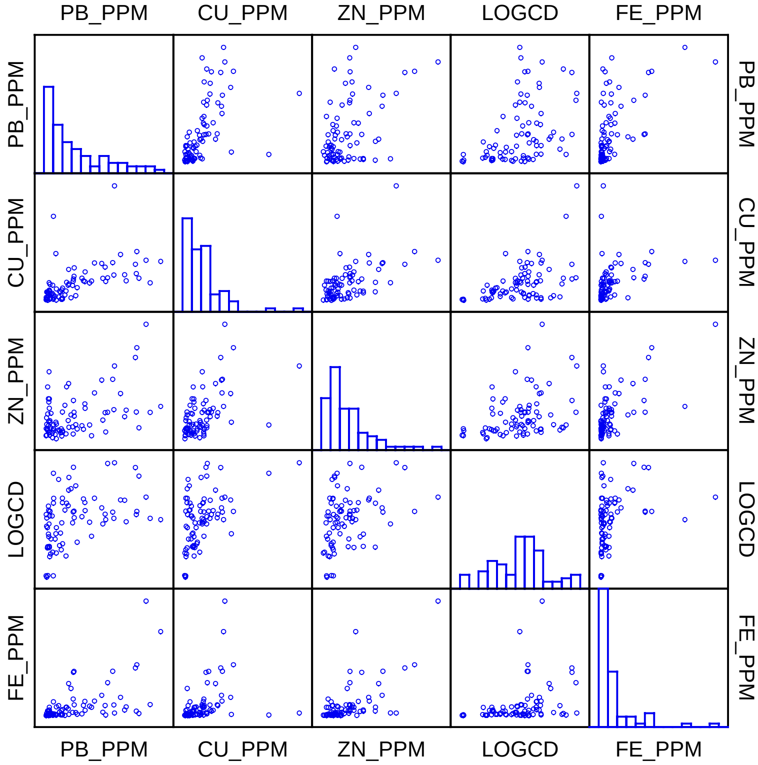

Fig. 1. Plot of correlations between the metals values in the set of bones analysed n=80 (transformed logarithmic values for Cd).

correspond to the transformed logarithmic data. Figs. 8–12 present the mean values for each of the 7 epochs in which the bones were classified together with the trend lines.

4. Discussion

Bone tissue has frequently been used to study accumulated exposure to metals, since it is the main

Table 5
Correlations between the metals in bones corresponding to Epoch B from the Bronze Age to the 18th century

		Lead	Copper	Zinc	Cadmium	Iron
Lead	Pearson's Coeff.	1.000	0.525**	0.604**	0.237*	0.571**
	Bilat. Sig.		0.000	0.000	0.052	0.000
Copper	Pearson's Coeff.		1.000	0.593**	0.501**	0.327**
	Bilat. Sig.			0.000	0.000	0.007
Zinc	Pearson's Coeff.			1.000	0.330**	0.723**
	Bilat. Sig.				0.006	0.000
Cadmium	Pearson's Coeff.				1.000	0.196
	Bilat. Sig.					0.109
Log Cd	Pearson's Coeff.	0.356**	0.457**	0.446**	0.689**	0.303*
	Bilat. Sig.	0.003	0.000	0.000	0.000	0.012

N=68.
* The correlation is significant at the 0.05 level (bilateral).
** The correlation is significant at the 0.01 level (bilateral).

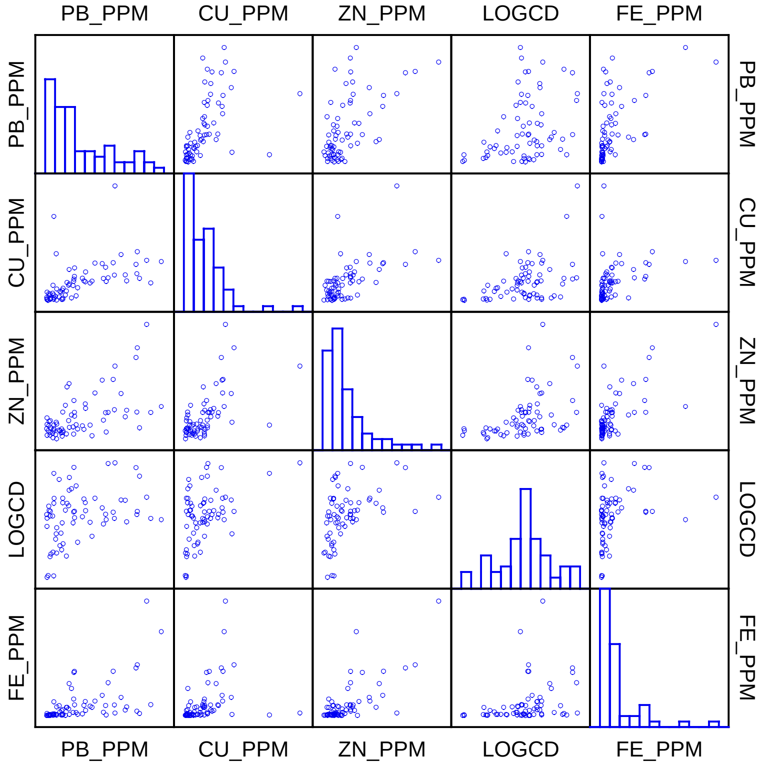

Fig. 2. Plot of correlations between the metals values in the analysed bones from historical epochs n=68 (transformed logarithmic values for Cd).

mineral component of the human body (Sanín et al., 1998). The concentration of lead in bones in different periods in history has been reported by diverse authors (Drasch, 1982; Ericson et al., 1991; Baranowska et al., 1995), more than 90% of the bodily load of lead being found in the skeleton, where replacement is slow (Bergdahl et al., 1998). This period varies depending on the part of the bone considered, ranging between 16 and 27 years. The concentration of lead in bone thus reflects long-term exposure (Sanín et al., 1998).

Table 6
Level of significance for the Kruskal–Wallis test (factor Epochs 1 to 4) carried out for lead, copper, zinc and iron and of the ANOVA (factor Epochs 1 to 4) carried out for the transformed logarithmic data for cadmium

Metal	Lead	Copper	Zinc	Cadmium	Iron
Test	Kuskal–Wallis	Kuskal–Wallis	Kuskal–Wallis	ANOVA	Kruskal–Wallis
Level of significance	0.000	0.002	0.003	0.001	0.253

In this study, we analysed the content in lead, copper, zinc, cadmium and iron in a total of 80 samples corresponding to 38 individuals, 30 of which were found at archaeological excavations carried out in the city of Cartagena. Four samples belong to an individual from the Neolithic period, 68 of the samples correspond to historical periods ranging from the Bronze Age to the 18th century, and 8 samples are the bone remains of present-day individuals who had died in the 21st century, and which were made available by the Anatomical Forensic Institute of Cartagena. The number of samples studied was conditioned by the bone remains existing at the Municipal Archaeological Museum. The remains are likewise lacking in socio-demographic data and correspond to the remains of persons who died and were buried in the city, and who we consider probably lived most of their life in this area. Bone remains from historical periods are studied and classified at their corresponding excavations and some are detailed in the study carried out in the city in the year 1990 (Portí Durán et al., 1999).

Table 7
Comparison of the mean values of metals according to Epochs 1 to 4

	Epochs 1 to 4a–b	N_a	N_b	t	Level of significance
Log Pb	1–2	4	25	−5.921	0.001
	1–3	4	43	−5.219	0.005
	1–4	4	8	−1.471	0.226
	2–3	25	43	1.151	0.255
	2–4	25	8	7.263	0.000
	3–4	43	8	7.830	0.000
Copper	1–2	4	25	−3.759	0.001
	1–3	4	43	−1.919	0.118
	1–4	4	8	−1.106	0.335
	2–3	25	43	2.924	0.007
	2–4	25	8	3.659	0.001
	3–4	43	8	1.611	0.115
Zinc	1–2	4	25	−3.329	0.003
	1–3	4	43	−2.235	0.058
	1–4	4	8	−5.231	0.000
	2–3	25	43	2.263	0.031
	2–4	25	8	−0.962	0.345
	3–4	43	8	−4.082	0.002
Log Cd	1–2	4	25	−2.954	0.006
	1–3	4	43	−1.385	0.173
	1–4	4	8	−0.055	0.958
	2–3	25	43	2.437	0.016
	2–4	25	8	4.443	0.000
	3–4	43	8	1.961	0.056
Log Fe	1–2	4	25	−2.372	0.056
	1–3	4	43	−2.450	0.069
	1–4	4	8	−2.882	0.055
	2–3	25	43	0.213	0.832
	2–4	25	8	−0.218	0.829
	3–4	43	8	−0.647	0.521

Direct values were used for the metals copper and zinc and transformed logarithms for lead, cadmium and iron; distinct variances were observed in all cases except for cadmium. N: sample size.

The degree of conservation of the historical material is very good. All the samples are catalogued and archived in the storerooms of the Municipal Archaeological Museum. According to experts in the matter, they were found at excavations at different sites in areas that were used as burial sites following the uses and customs of each epoch, and which in some cases have subsequently been covered over by buildings.

The distribution of lead in the human skeleton is not homogeneous. The major differences between different bones are found in adolescence, when the lead from the vertebrae is greater than that found in the tibia, rib, ilium and cranium, these differences between bones tending to disappear with age (Wittmers et al., 1988). At the same time, as has already been reported (Sanín et al., 1998), the different degree of accumulation of metals in bones is a

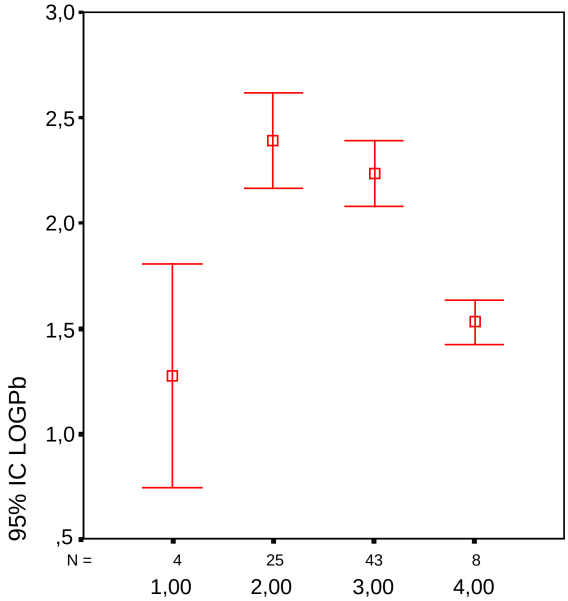

Fig. 3. Mean with a confidence interval of 95% for the logarithm of lead for Epochs 1 to 4.

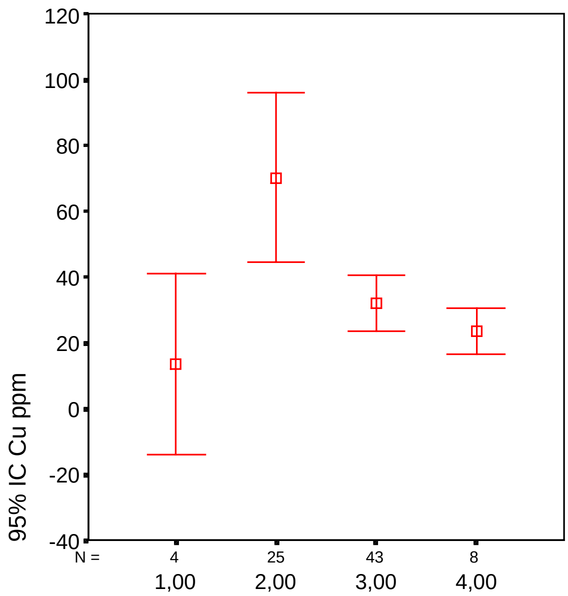

Fig. 4. Mean with a confidence interval of 95% for copper for Epochs 1 to 4.

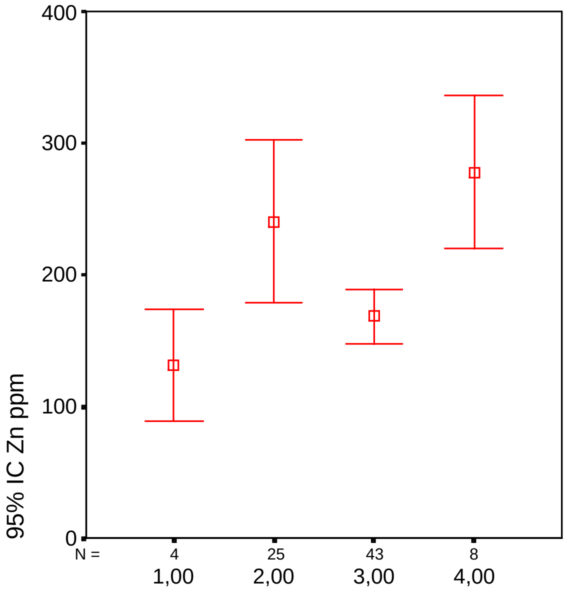

Fig. 5. Mean with a confidence interval of 95% for zinc for Epochs 1 to 4.

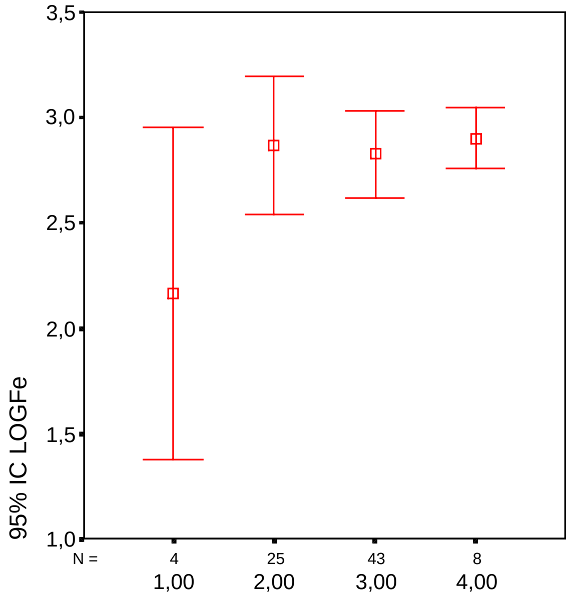

Fig. 7. Mean with a confidence interval of 95% for the logarithm of iron for Epochs 1 to 4.

function of the type of bone, the area of this bone and its state of ossification. Selection of the bone to analyse may be an important factor, but in archaeological samples the choice of bone type may be very limited. To avoid errors in the extrapolation of results, whenever possible we analysed different bone pieces from the same individual. We consider that when analysing different bones from the same individual, we minimise the differences that may be attributable to the bone type.

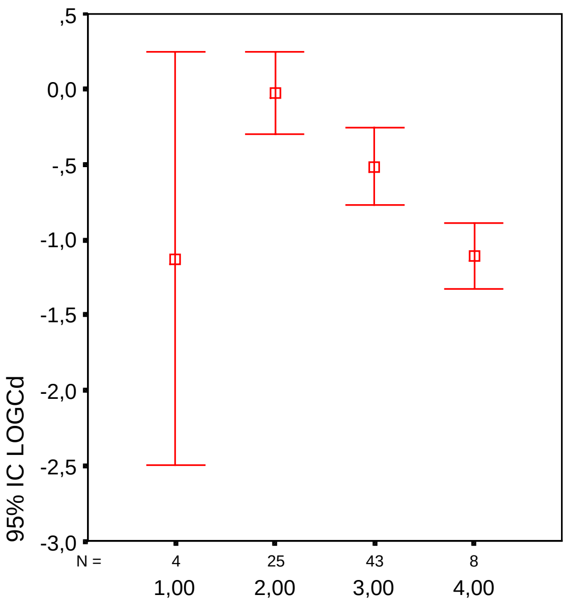

Fig. 6. Mean with a confidence interval of 95% for the logarithm of cadmium for Epochs 1 to 4.

The passing of time after burial determines the loss in organic matter, the content of which was substantial in the bones of present-day individuals. Therefore, in order to avoid the variation that this factor might introduce in the analytical results, all the samples were calcined, thus eliminating the existing organic matter.

The maximum weight of bone used was 0.5 g. After calcination, the samples were dissolved in an acid medium (nitric and perchloric acid) using Suprapur quality reagents in an open atmosphere. The contents in lead, copper, zinc, iron and cadmium were then quantified against standards by anodic stripping voltammetry or atomic absorption spectrometry on the basis of the sensitivity ranges and detection limits of each of the techniques and the metals content in the bones. One out of every ten processed samples corresponded to a certified bone standard.

After the initial data exploration, and since the distributions of copper and zinc fitted neither the normal nor the lognormal distribution, whereas those

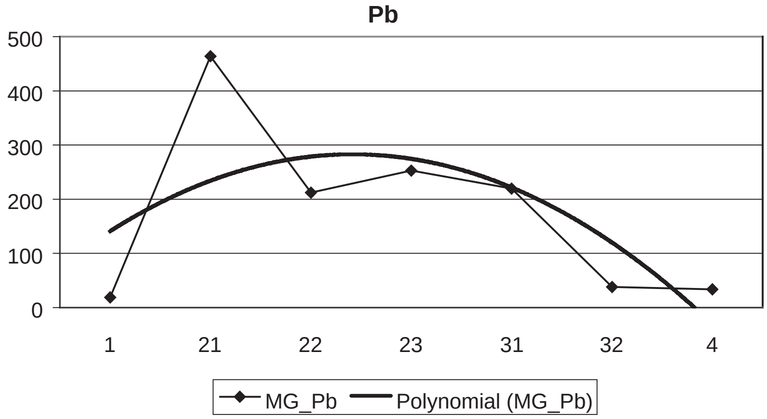

Fig. 8. Value of the geometrical mean of lead for the 7 epochs and the trend line.

of lead, cadmium and iron fitted the lognormal distribution, we decided to work with real values for the former metals in the statistical treatment of the data. However, we worked with both direct data as well as transformed logarithmic data for the latter metals.

Table 2 shows the statistics, arithmetic mean, standard deviation, minimum and maximum values and the sample size for all the metals for the different groupings carried out on the basis of the dating of samples and for the totality of analysed samples. Table 3 shows the geometric mean and the standard geometrical deviation for lead, cadmium and iron, since the measures of the central tendency and the dispersion of data are better in the lognormal distributions.

Diverse authors have highlighted the fact that the modifications induced by anthropic activity are not an exclusive product of modern times (Settle and Patterson, 1980; Nriagu, 1996). Since the discovery of mining and metal processing, a close relation has existed between pollution and human activities (Martínez Cortizas et al., 1991). It would appear that the natural thresholds of certain heavy metals such as lead or copper began to increase with the discovery of metals, acquiring a historical peak during the flourishing of the Roman Empire, at least 2000 years before the present day (Settle and Patterson, 1980; Nriagu, 1996).

Of the metals analysed in this study, copper, zinc and iron are essential elements that must be administered in the diet, since otherwise pathologies

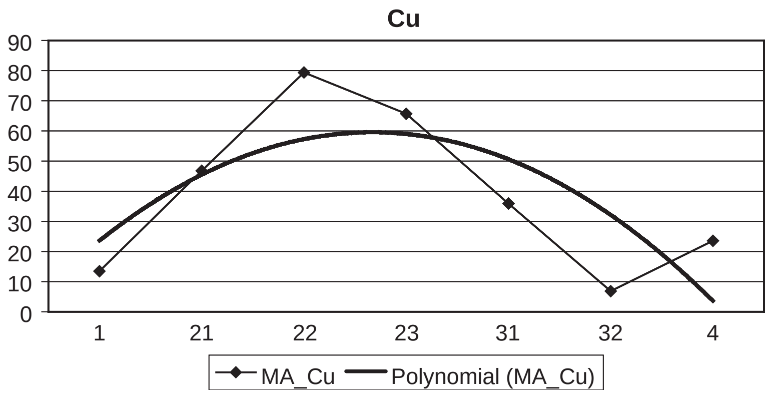

Fig. 9. Value of the arithmetical mean of copper for the 7 epochs and the trend line.

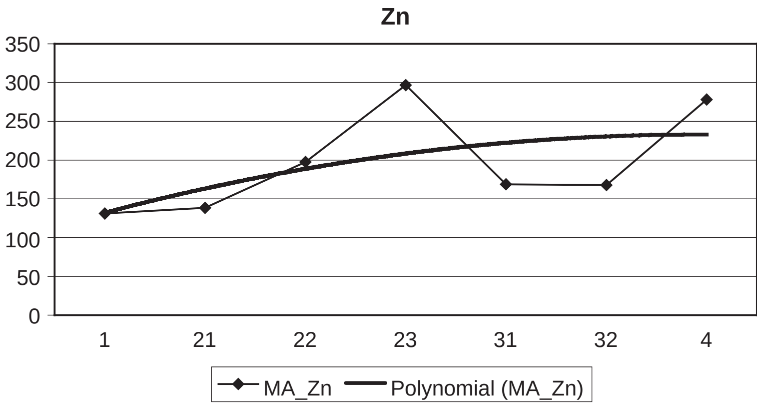

Fig. 10. Value of the arithmetical mean of zinc for the 7 epochs and the trend line.

develop. Although both copper and zinc may give rise at high concentrations to the appearance of acute and chronic intoxication (ATSDR, 1994; ATSDR, 2002), iron is the only non-vestigial heavy metal found in human beings. Only the pharmacological forms of iron are of toxicological interest, given the concentration of the metal, its availability and easy intake, cases of acute intoxication due to this metal having been reported (Rojano and Cepello, 1984). Lead and cadmium do not play any known beneficial role in the human organism and are considered toxic elements with acute and chronic effects and giving rise to diverse pathologies (ATSDR, 1999; ATSDR, 2000; Moreno Grau, 2003). The adverse effects of lead have been reported in cases of chronic exposure to low concentrations, above all in children (ATSDR, 2000).

The data reported by diverse authors in relation to the metals content in bones, both in archaeological remains and in present-day populations, are not homogeneous. The lead in mineralised tissue is not uniformly distributed, but rather tends to accumulate in the areas of the bone subjected to a more active process of calcification at the time of exposure (ATSDR, 2000). All authors consider bones to be a good indicator of chronic exposure, although the shortage of in vivo methods for its quantification limits their use, teeth being used more often in studies of the present-day population. In a classic treatise containing diverse scientific tables published in Geigy Documenta in 1965, the following values are reported as normal in the chemical composition of the human skeleton: 18.8 ppm (μg/g) of lead in

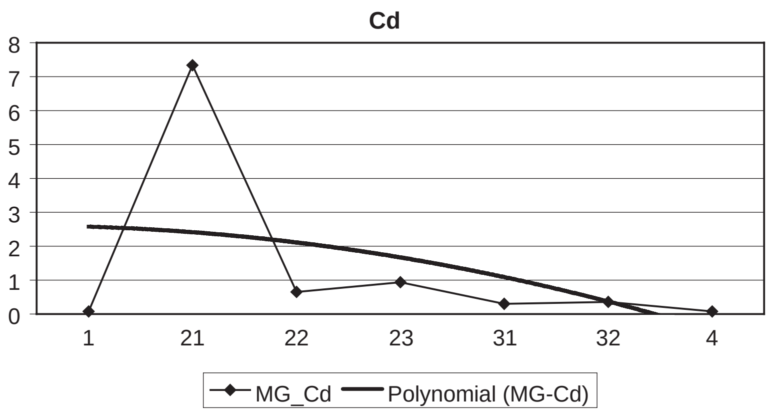

Fig. 11. Value of the geometrical mean of cadmium for the 7 epochs and the trend line.

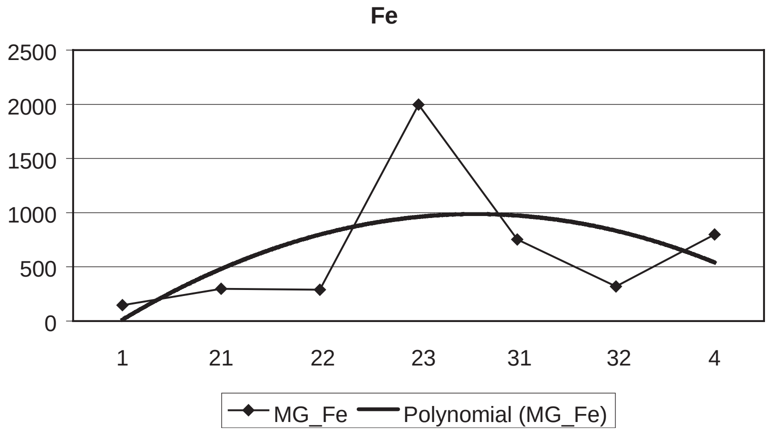

Fig. 12. Value of the geometrical mean of iron for the 7 epochs and the trend line.

the long bones and 4.7 ppm in the ribs; while for copper the reported values are 11.9 ppm in the long bones and 4.1 ppm in the ribs. For this latter metal, the ATSDR (2002) reports mean values in human bones of 4.2 ppm.

Jaworowski et al. (1985) present data on human bones from diverse excavations carried out in France, a finding that a very important change has taken place over time for both lead and cadmium, while the concentrations of zinc have remained more or less stable. As regards lead, the data reported in this study of the Neolithic period present very low values, below the detection limits of the technique employed (<0.1 ppm), increasing for the Bronze Age, with a maximum value of 36 ppm. Similar values to those of the Bronze Age are reported in the Roman period. There is a substantial increase in the concentrations of lead in bones from the year 1200 A.D., the maximum for the samples corresponding to the year 1500 A.D. (the highest value reported by researchers is of 280 ppm for lead). As regards present-day bones, these present mean values of 16 ppm for lead, ranging between 5 and 35 ppm. For cadmium, a brusque increase in concentrations is reported from the year 1800 A.D., with values of this metal ranging from <0.05 to 8 ppm, the higher values corresponding to present-day individuals (Jaworowski et al., 1985).

In a study carried out on present-day bone samples belonging to 35 individuals who had died in 1993 and had resided in an industrial district of Poland, Baranowska et al., 1995 found mean values of 57.55 ppm of lead, 0.59 ppm of copper, 115.13 ppm of zinc and 0.53 ppm of cadmium. On the other hand, Bergdahl et al., 1998, in a study on the lead concentration in bones of a population of workers at a secondary lead smelting foundry with a sample of 77 exposed workers and 24 control subjects, found values that ranged from <D.L. to 193 ppm for the exposed population and from <D.L. to 36 ppm for the controls in the samples of tibial bone; and between <D.L. and 248 ppm for the exposed workers and from<D.L. to 61 ppm in the controls, when the samples came from heel bone.

In the complete set of samples that we studied (Table 1), individual number 15 is dated within the Neolithic, with 4 available samples, low values of lead being found in this individual's bone remains, an arithmetic mean of 23 ppm (geometric mean, 18.8 ppm). We analysed two samples from individual number 14, corresponding to the Bronze Age, Epoch 2.1, the mean value for lead being 578.7 ppm (geometric mean, 463.9 ppm). Of the individuals classified within Epoch 2.2, corresponding to the Roman period, 11 samples were analysed, finding a mean value for lead in these samples of 369.9 ppm (geometric mean, 212.2 ppm). 12 samples correspond to the Byzantine period, Epoch 2.3, in which we found a value of the arithmetic mean for lead of 459.4 ppm (geometric mean, 253 ppm). A total of 37 samples were analysed from the Islamic period, Epoch 3.1, for which the mean value for lead is 316.5 ppm (geometric mean, 219.6 ppm). 6 samples correspond to the Modern period, Epoch 3.2, with a mean value for lead of 82.6 ppm (geometric mean, 38.2 ppm), and

8 present-day samples show a mean value for lead of 35.1 ppm (geometric mean, 33.7 ppm).

When we group together the individuals from the Bronze, Roman and Byzantine periods into one single group (2) and those from the Islamic and Modern periods into another (3), the mean value for lead found in the first regrouping (2) is of 429.4 ppm (geometric mean, 245.8 ppm), and for the second (3) of 283.8 ppm (geometric mean, 172 ppm).

The results for the lead found in the bones of the Neolithic, Bronze, Roman and Byzantine periods are much higher than those reported by Jaworowski et al., 1985, in archaeological bones from France in the same periods. For the epoch that corresponds in our samples to the Islamic period, although the mean values given by the aforementioned authors are lower, in some samples they are situated at higher values, up to 280 ppm. For the samples from the 18th century, the values in both studies are more similar: a mean of 61 ppm for the French remains and 82.6 ppm in the bones from Cartagena. The same occurs with the present-day samples, in which the French bones present a mean value of 16.9 ppm and those of Cartagena 33.7 ppm.

For present-day bones, the values of lead that we found in the bones are situated within the order of magnitude of the values reported in the studies of Baranowska et al. (1995) and Bergdahl et al. (1998) and within the range of normal values for unexposed individuals reported by authors such as Kowal et al. (1989) (15–50 ppm).

These results appear to indicate a greater exposure to lead in the populations who dwelt in our geographic area in comparison with those situated in France. This is easily justified by the intense mining of the ore of the studied metals existing in our area. In fact, the contribution of these metals to the settleable atmospheric aerosol is still very important today, our research group having reported in previous studies that the soil in the area is a source of heavy metals (Moreno-Grau et al., 2002).

To examine further the differences between these studies, the samples of soil analysed in the vicinity of the French excavations present contents in lead of 19 ppm for the Neolithic site, between 6 and 10 ppm for the sites dated between the years 600 and 800 A.D., and of 35 ppm for the soils adjacent to the sites dated between the years 1400 and 1600 A.D. (Jaworowski et al., 1985), whereas the soils in Cartagena presents mean values in lead ranging between 1260 ppm in unpolluted areas to 149920 ppm in the areas in the vicinity of activities related to the working of lead, with an overall mean for municipal soils of 40170 ppm (Martínez-García et al., 2001). A similar situation is found in the case of cadmium, another element analysed in the soils in both studies. For the French soils, values of cadmium of between 0.3 and 1.3 ppm were found (Jaworowski et al., 1985), whereas in the soils in Cartagena mean values of between 14 ppm were found for unpolluted areas and 9300 ppm in the areas in the vicinity of activities related to the working of lead, with an overall mean for municipal soils of 3280 ppm (Martínez-García et al., 2001).

On the other hand, in prehistoric times the contribution of lead to settleable particulate matter was 2 ng/cm^2 year (Settle and Patterson, 1980), whereas the present-day deposition in the oceans is 60 ng/cm^2 year and 270 ng/cm^2 year in terrestrial ecosystems. The lead content in the suspended atmospheric aerosol in prehistoric times presented values of 0.04 ng/m^3 and of 800 ng/m^3 in urban settings in the United States in 1980. The aforementioned authors also indicate that in Sierra Nevada (California), the deposition of lead is 130 ng/cm^2 year and the lead content in the atmospheric aerosol is 10 ng/m^3.

In Cartagena, the total deposition of lead in the settleable particulate matter in the years 1986–1987 ranged between 2550 ng/cm^2 year in a non-industrialized area and 135,050 ng/cm^2 year in the industrialized area of the city (Moreno-Grau et al., 2002). As regards the lead content in the suspended atmospheric aerosol (TSP), the mean value for lead between the years 1991 and 1998 was situated between 250 ng/m^3 in the non-industirilized area of the city and 1010 ng/m^3 in the vicinity of a lead foundry (Moreno-Grau et al., 2000).

For copper, it is estimated that 97% of what is released into the atmosphere is contained in the soil, whereas 0.04% is found in the air. As it is a normal component of the earth's crust, its primary natural source is the dust transported by the wind, as well as forest fires, volcanoes, biogenic processes and the marine aerosol. As anthropogenic sources, we have the production of non-ferrous metals, timber, iron and steel production, waste incineration, etc. It is estimated that the global anthropogenic emission of copper is $35 \cdot 10^6$ kg/year, along with natural emissions of $28 \cdot 10^6$ kg/year. The copper concentrations found in air samples are located within the range 0.02 to 10 μg/m^3, with a mean value of 0.38 μg/m^3. The atmospheric deposition of this metal in rural areas of the United States is located within the range 510 to 5330 ng/cm^2 year. Amounts of between 0.01 and 182,000 ppm have been reported in soils, with a mean value of 0.103 ppm (ATSDR, 2002).

The mean values of the concentrations of copper in the atmospheric aerosol of Cartagena for the period 1991–1997 vary between 0.03 μg/m^3 in areas distant from industrial activity and 0.05 μg/m^3 in the vicinity of an electrolytic zinc plant (Moreno-Grau et al., 2000). The mean values of this metal in the settleable particulate matter varied between 3650 and 14600 ng/cm^2 year (Moreno-Grau et al., 2002) The mean values of copper found in the soil of the Municipality were 21.1 ppm (Martínez-García et al., 2001).

Zinc is the other element that is normally found in the earth's crust, being released into the atmosphere from both natural as well as anthropogenic sources. However, the anthropogenic contributions for this metal are much more substantial. Of these contributions, the most important come from the mining and working of this metal and the use of commercial products that contain zinc. At a global level, releases to the soil are probably the main sources of zinc in the environment. The concentrations of zinc in the air are relatively low and constant, except in the vicinity of the sources of this metal, such as refining plants. In the United States, values are usually situated below 1 μg/m^3; mean values of this metal in urban areas in said country may be 0.127 μg/m^3, ranging between 0.027 and 0.500 μg/m^3. Zinc is found in the soil in concentrations of between 10 and 1300 ppm. In the soil map of the United States, values have been found for cultivated soil ranging from below 5 up to 400 ppm, and from below 10 up to 2000 ppm in non-cultivated soil (ATSDR, 1994).

The mean values of the concentrations of zinc in the suspended atmospheric aerosol of Cartagena for the period 1991–1997 vary between 1.36 μg/m^3 and 2.10 μg/m^3, depending on whether the sample was taken in an urban area or in an industrial area, in the vicinity of an electrolytic zinc plant (Moreno-Grau et al., 2000). The total deposition of this metal in the period 1986–1987 was 124,100 ng/cm^2 year in the urban area of the city and 580,350 ng/cm^2 year in its industrial zone (Moreno-Grau et al., 2002). Mean values of 176.4 ppm have been reported in the soil of the Municipality (Martínez-García et al., 2001).

Iron is the fourth most abundant element in the earth's crust, after aluminium, silicon and oxygen. As stated above, it is an essential micronutrient for man and presents a minimum risk to health, and is not included within the list of hazardous pollutants in the most important regulations in Europe or the United States (Kirk and Othmer, 2001). In Spain, the metal iron has no environmental limit value for occupational exposure, although iron oxide (III), soluble iron salts and iron dicyclopentadienyl and pentacarbonyl do (MTAS, 2003). It is efficiently recycled by the organism, excretion mechanisms not existing, being lost via exfoliation of the mucus, skin or hair loss or via haemorrhages. Iron absorption by the human body is precisely regulated on the basis of existing needs (Kirk and Othmer, 2001). The values of iron in the suspended atmospheric aerosol that we have found reported in the literature range between 0.216 μg/m^3 (0.008–1.201 μg/m^3) in the southeast of England (Yaaqub et al., 1991) or 0.4 μg/m^3 (0.0–6.84 μg/m^3) in Oporto (Pio et al., 1998) and 3.57 μg/m^3 (0.95–9.94 μg/m^3) in Seville (Usero et al., 1988). This element has been chosen as an indicator of the contribution of the earth's crust (Usero et al., 1988 and Yaaqub et al., 1991). As regards the iron content in the atmospheric deposition, Tanner and Wong (2000) report 6070 ng/cm^2 year for Hong Kong, 96,700 ng/cm^2 year in Malaysia, 14,000 in Massachusetts, and 11,100 ng/cm^2 year in the North Sea.

The iron content in the settleable particulate matter in Cartagena for the period 1986–1987 ranged between 284,700 ng/cm^2 year in the urban area of the city and 1,040,250 ng/cm^2 year in the industrial zone (Moreno-Grau et al., 2002).

As can be seen, the content in the metals under study is very high, both in the suspended atmospheric aerosol as well as in the settleable particulate matter and in the soil of Cartagena. Although we do not have data from historical epochs, the natural exposure of the inhabitants of our area to these metals must have been much greater than we have reported for the samples from the end of the 20th century, when environmental control measures have limited emissions from industrial sites. Even at times of scarce mining-metalworking activity, the exposure of the population must have been substantial, as a result of the high content of these metals in the soil of the region, which is one of the reserves of the ores of these metals in Europe (Román, 1987), which act as a reservoir and source of these metals for the aerosol. The region is also semi-arid, with scant vegetation, thus favouring the contribution of the soil to the aerosol due to the effect of the wind.

All the analysed metals are found linked to the mining-metalworking activities carried out in the area, and so the analysis of the correlation between these may help identify whether the origin of these metals in bones is the same. In fact, several authors have used correlation analysis as a first approximation in the development of receptor models aimed at establishing the contributions of the sources of pollution or at establishing the route of entry (Gradjean et al., 1992; Scheff, 2000).

To carry out the correlation analysis, we employed the values of the variables lead, copper, zinc, cadmium and iron, and the transformed logarithms of the variable cadmium. The results are presented in Table 4, for the complete set of analysed samples, and 5, for those corresponding to Epoch B. Only those transformed logarithmic variables with which a significant improvement in the correlations was obtained are included. Figs. 1 and 2 present the graphs of the corresponding correlations. We can observe that there are positive correlations at a level of significance of 99% between the metals lead, copper, cadmium (transformed logarithmic variable) and iron for the complete set of samples, except for the relation between iron and cadmium (transformed logarithmic variable), in which the level of significance is 95%. We thus consider the metals to share the same origin in bones, i.e. their presence in bones is the consequence of the important contribution of the same ingress routes for all of them, inhalation of atmospheric aerosols and ingestion as a result of diverse contributions in the diet.

One of the aims of this study is to establish the possible variation in the concentrations of the analysed metals throughout the periods in which the samples were classified, using as a factor the classification in four epochs: 1; Neolithic, 2; Bronze Age to the Byzantine period, 3; from the Islamic period to the 18th century, and 4: present-day remains.

With the aim of ascertaining the statistical significance of the differences between the mean values of the metals under study grouped into four groups according to the factor epoch, the Kruskal–Wallis test was carried out for the distributions that do not fulfil the basic assumptions of analysis of variance, i.e. a normal distribution and homogeneity in variances in the groups associated with the factor, and ANOVA for cadmium, which does satisfy these assumptions. The results are given in Table 6, in which it can be seen that the level of significance of the ANOVA carried out for the transformed logarithmic variable of cadmium shows a mean value of 0.05, indicating the existence of differences with statistical significance for the factor epoch introduced in the analysis. The Kruskal–Wallis test carried out for the remaining metals shows differences with statistical significance for lead, copper and zinc (n.s. <0.05), no differences existing between the groups for iron (n.s. 0.253).

In the tests comparing the mean values (Table 7), it can be seen that for lead the bone remains from Epochs 1 and 4 and 2 and 3 constitute homogeneous groups (transformed logarithmic data), the remains from Epochs 2 and 3 (from the Bronze Age until the 18th century) presenting higher values. For copper, the remains from Epoch 2 show the highest content, the remaining epochs constituting a homogeneous group. For zinc, the bones from Epoch 1 have a lower mean value, although the difference is not statistically significant to those from Epoch 3. The present-day bones present the highest mean value, but the difference with respect to those of Epoch 2 is not statistically significant.

For cadmium, Epochs 1 and 4 present the lowest mean values, Epochs 1, 3 and 4 constitute a homogeneous group. The highest mean value for this metal was found for Epoch 2, and is statistically different. For iron, Epoch 1 presents the lowest mean value, although there are no significant differences between the different epochs for this metal. Figs. 3–7 show the mean values with a 95% confidence interval for the mean, grouped together for this factor (epoch in 4 groups) employing transformed logarithmic data for lead, cadmium and iron and direct data for copper and zinc, and may be useful when examining the behaviour of these metals.

The obtained results show that the mean values of all the analysed metals present the lowest values for the samples corresponding to Epoch 1, the Neolithic individual. These remains present similar values to those found in present-day remains in the case of lead, copper, cadmium and iron. In the case of zinc, the Neolithic remains present similar values to those from Epoch 3 (Islamic period to 18th century). It should be noted that in the case of lead, there exists evidence that indicates that the levels in European populations have decreased substantially throughout the 20th century as a consequence of the improvement in hygienic and environmental control measures (Jaworowski et al., 1985). On the other hand, deficiencies in iron and calcium increase the absorption of lead and aggravate pica habits (eating earth, sucking one's fingers, etc.) (ATSDR, 2000), and hence this factor may have an influence in undernourished individuals.

The results found for lead coincide with the evolution of its production throughout history (Settle and Patterson, 1980; Boeckx, 1986), who report that global production of lead was around 160 tons/year some 4000 years ago (2000 B.C.), 10,000 tons/year 700 years B.C., the period when silver coins began to be minted, reaching some 80,000 tons/year with the flourishing of the Roman Empire, around 2000 years ago. Production declined during the medieval epoch, once more increasing with the Industrial Revolution from 100,000 tons/year in 1700 to 1,000,000 tons/year in 1930 and some 3,000,000 tons/year in 1980. Our results also fit the data for this metal found in peat bogs in Galicia (Spain), which are correlated with anthropic atmospheric pollution (Martínez Cortizas et al., 1991). Environmental pollution caused by heavy metals is thought to have commenced with the domestication of fire, but it is from the discovery of mining and metal processing onwards when a close relation has been established between atmospheric pollution and human activities (Nriagu, 1996).

When an trend analysis is carried out for the values of the metals classified in the seven epochs in which the remains were grouped (Figs. 8–12), it can be seen that lead presents an increasing tendency between Epochs 1 and 2.3 (Neolithic and Byzantine), its concentration in bones subsequently decreasing. The peak of the trend is found between Epochs 2.2 and 2.3 (Roman and Byzantine). Copper and iron behave similarly, but the peak of the trend is found somewhat later on, especially for iron, since the maximum value of the plot is found in period 2.3 (Byzantine) and that of iron in period 3.1 (Islamic). The concentrations of zinc show an increasing trend over the different historical periods under study, while cadmium behaves in the contrary manner, the slope of the trend line is negative, its concentration in bones decreasing over time.

The samples with which this study was carried out range from the Neolithic to the 18th century, along with 8 present-day samples. Therefore, we do not have material in which we might test the effect that the Industrial Revolution may have had on the concentration of the analysed metals in bones. However, the obtained results clearly show that the use of metals by man led to a very substantial increase in the concentration of extraneous metals in the composition of human tissues, xenobiotic compounds of lead and cadmium, and that the concentrations of these metals vary over time. An increase was produced for lead in the Bronze Age that was maintained during the Roman and Byzantine periods, until decreasing in the 18th century. This data would coincide with the decline in mining activity in this period, which was described for the city of Cartagena in Introduction. It also coincides with the data published by Martínez Cortizas et al. (1991) in relation to the Northeast of the Peninsula, who agrees with the authors that it would seem logical to think that the inhabitants of these periods would not fail to take advantage, if only to supply their own needs, of an existing sector in the area. Not necessarily in the form of large mines, but as a resource for the elaboration of piping, stamps, ballast, etc. The presence of Byzantium in our region has also been included in this reflection, as it would seem reasonable to link this presence with the exploitation of the mines in Cartagena, with profits not only from lead but also from the silver present in the silver-bearing galenite in the area. Thus, Settle and Patterson (1980) report that the desire to obtain silver was what led to the obtaining of lead in remote times, 400 parts of lead being obtained as a by-product for each part of silver smelted from ore. A by-product for which numerous immediate applications were found: coins, piping, glazes, food additives, etc.

5. Conclusions

1. The data obtained from the studied samples allow us to conclude that after the use of metals by man, an increase was produced in the concentrations of lead, copper, zinc, cadmium and iron accumulated in the bones.
2. The behaviour of the accumulation of the studied metals over time varied on the basis of whether the metal in question was xenobiotic or whether it was essential in character, high concentrations of the latter remaining in the present-day bone remains were studied. However, the concentrations of the xenobiotic metals reached a peak then decrease to the values found in the samples that belong to present-day individuals. We consider this result to be linked to the pollution control measures implanted during the last decades of the 20th century.
3. The analysis of correlation carried out allows us to conclude that the studied metals in bone remains are a consequence of the important contribution of the same entry routes for all of these, presumably both via inhalation of atmospheric aerosols as well as ingestion as a result of diverse contributions in the diet.

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