



# Macro and micro elements in traditional meals of Mediterranean diet: Determination, estimated intake by population, risk assessment and chemometric analysis

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## ABSTRACT

The aim of the study was to estimate the dietary exposure of the main macro and micro elements from meals typically consumed by the Mediterranean population, in particular Sicilian by samples prepared and cooked prior to analysis. A total of 57 typical Mediterranean meals samples were analysed for 4 macro elements: Calcium (Ca), Potassium (K), Magnesium (Mg) and Sodium (Na) and 13 micro elements: Aluminium (Al), Arsenic (As), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Copper (Cu), Iron (Fe), Mercury (Hg), Manganese (Mn), Nickel (Ni), Lead (Pb), Zinc (Zn) and Vanadium (V) by inductively coupled plasma - mass spectrometry and atomic absorption spectrometry. Mercury concentration was quantified directly on the food samples by using the DMA-80 (Direct for Mercury Analysis). Daily Estimated Element Intake was calculated for essential element and assessment of health risk associated with intake of investigated elements was performed. In particular, analysed samples supply from 3.7% to 62% of sodium, 0.05–19% of potassium, 0.1–3.2% of calcium, 1.6–16% of magnesium, 2.1–37% of iron, 0.7–48% of zinc and 0.98–339% of chromium. Some of the foods prepared are lacking in all macroelements (Na, K, Ca, Mg). The quantity of macroelements taken from a single portion of 100 g of several food, compared to the recommended quantities, is low, but it should be considered that on average in one day (breakfast, lunch and dinner), undoubtedly, the inhabitants of the Mediterranean area consume much higher quantities for a total of 500–1000 g, therefore, this study confirmation for the populations concerned, the low probability of nutritional or health risks due to the consumption of foods, indeed, the consumption of Mediterranean dietary foods, has not shown particular risk assessments from heavy metals.

## 1. Introduction

Cultural background can have significant influence on food and beverage choices. The term, Mediterranean Diet (MedDi), originally proposed by Ancel and Margaret Keys in the famous book *How to eat well and stay well: The Mediterranean way* (Keys Ancel, Keys Margaret, 1975), published in 1975, in the intentions of the authors, had to provide for eating habits and related culinary recipes of the working and middle class of the regions of the European coast of Mediterranean. Today, this type of diet is a healthy eating pattern, among people living in the Mediterranean area, that practically consists in the abundant use of extra virgin olive oil, high consumption of foods of vegetable origin (vegetables, fruit, legumes and dried fruit), from moderate to high

consumption of fish, whole grains and red wine (mainly used during meals) and a limited consumption of simple sugars (sweets, sugary drinks, etc.), red meats, cold cuts, milk, butter (Keys Ancel, Keys Margaret, 1975).

Several researches have studied the correlation between implementation of Mediterranean diet and the appearance of breast cancer, in particular, some studies indicate a protective role of this eating habit (Castello et al., 2014; Martínez-Gonzalez et al., 2017; Turati et al., 2018). Some epidemiological research showed that the risk of death due to cardiovascular disease is higher in overweight individuals. In this case, the dietary pattern with the largest body of evidence of health benefits is the one traditionally followed by the inhabitants of some Mediterranean countries (Dominguez et al., 2023). In addition, the

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**Table 1**  
Description of the samples.

N°	Dishes name	Description and ingredients
1	Chocolate cake	It is a classic and timeless Italian dessert made with chocolate or cocoa
2	Caponata	A typical Sicilian sweet and sour starter made with fried eggplant, tomato, onion, olives, celery, capers, vinegar, sugar.
3	Roast chicken with aromas	It is chicken cooked at higher oven temperature flavoured with lemons, garlic and rosemary
4	Boiled potatoes	Potatoes cooked in hot water
5	Pasta al forno Baked Pasta	A typical dish of Palermo's cuisine. it is a pasta seasoned with meat sauce, cheese, ham and, in some cases, boiled egg. The final cooking is done in the oven where it takes the form of a timbale.
6	Pasta with salmon	Pasta with smoked salmon and cream
7	Portulaca salad	Green salad with portulaca (a spontaneous edible plant), olive oil and salt.
8	Spaghetti carbonara	Spaghetti, fried in a pan, with bacon and egg
9	Boiled meat (muscle)	Boiled veal seasoned with oil and lemon
10	Cream of pea soup)	It's a creamy, velvety smooth soup that's made with peas and vegetable broth.
11	Boiled carrots	Carrots are cooked in boiling water for 5–10 min. They taste tender and savoury sweet
12	Boiled zucchini	Zucchini are cooked in boiling water for 5–10 min.
13	Omelette with courgette flowers	Omelette of eggs and pumpkin flowers
14	White bread	Type 0 flour bread
15	Cereal bread	Wholemeal flour bread containing cereals
16	Stewed peppers	A typical Mediterranean dish consisting of red and yellow pepper, potatoes, tomatoes and onions. All ingredients are stewed in a pan.
17	Tomato tripe	Beef tripe parts from all three edible stomachs of the cow with tomato, mint, white wine and pecorino cheese
18	Spaghetti tomato and eggplant	Simple, classic Sicilian pasta dish with fried eggplant, tomato sauce, salted Ricotta cheese and basil
19	Red peppers with potatoes	With its origins in Calabria, is an easy recipe for Italian slightly charred peppers and creamy, crispy potatoes
20	Boiled broad beans	Broad beans are cooked in boiling water for 15–20 min
21	Pizza	Most popular Italian dish consisting of a flattened disk of bread dough topped with some combination of olive oil, oregano, tomato, olives, mozzarella and many other ingredients
22	Roasted aubergines	Roasted aubergines flavoured with olive oil and Mediterranean herbs like mint, basil, oregano, rosemary
23	Peperoni rossi agrodolce	Sweet and sour peppers with tomatoes, onion, sugar, vinegar, salt, olive oil
24	Juvenile fish balls	Juvenile fish balls with Mediterranean fish, eggs, flour and parsley
25	Sweet and sour pumpkin	Sicilian-style sweet and sour pumpkin is a simple and rustic side made with fried pumpkin, onion, garlic, olive oil, salt, sugar, vinegar and mint
26	Green salad	It is a salad composed of leafy vegetables such as lettuce varieties, spinach, or rocket
27	Dolphinfish 1 with caponata sauce	In Sicily, Mahi Mahi is called Capone. In this recipe is served with caponata sauce with olives, capers, tomato, onion, celery, vinegar and sugar
28	Breaded chicken birbe	small piece of deboned chicken meat that is breaded or battered, then deep-fried or baked
29	Pasta with basil pesto 1	Pesto is one of the most popular pasta toppings in Italy made with fresh basil leaves, pine nuts, garlic, salt, Parmesan cheese, and olive oil.
30	vegetable minestrone	Italian vegetable soup made with seasonal vegetables and greens
31	Meat sauce (ragù)	Ragù is a meat-based sauce, typically served with pasta. Ragù is a sauce made from tomatoes and ground or chopped meat, which is cooked for a long time.
32	Pasta with zucchini	Pasta with roasted or fried zucchini, olive oil, garlic and mint.

**Table 1 (continued)**

N°	Dishes name	Description and ingredients
33	Grated yellow peppers	Roasted yellow peppers flavoured with olive oil and Mediterranean herbs like mint, basil, oregano, rosemary
34	Mussels pasta in tomato sauce	Fresh seafood pasta made with fresh mussels cooked and smothered in a tomato sauce with tomato, garlic, parsley, olive oil.
35	Pasta sauce and anchovies	Anchovies sauce is made with anchovies, garlic, oregano, tomatoes sauce. The dish is topped with toasted breadcrumbs
36	Rice salad	Italian Style Rice Salad is a chopped salad (tomato, carrots, olives, corn, peppers, onion) with perfectly cooked cold long grain rice mixed with homemade olive oil, lemon, herb and garlic dressing
37	Pasta pesto and tomatoes	Pesto is one of the most popular pasta toppings in Italy made with fresh basil leaves, pine nuts, garlic, salt, Parmesan cheese, and olive oil. Here we added cherries tomatoes
38	Arancine with meat	Sicilian arancini are one of Sicily's most popular street foods. Rice balls stuffed with ragù with peas, rolled in breadcrumbs and fried until crisp
39	Pasta with tuna sauce	Pasta served with tuna sauce made with tuna, chopped tomatoes, olives, red onions, capers, parsley, olive oil
40	Chicken salad	Chicken salad is any salad with chicken as a main ingredient and hard-boiled egg, mayonnaise, tomatoes, celery, onion, pepper, pickles.
41	Pasta with tenerumi	It's a soup with a particular ingredient <i>tenerumi</i> , which in Sicilian dialect refers to the sprouts and tender leaves of a variety of zucchini. Other ingredients are tomatoes, caciocavallo ragusano cheese, Extra-virgin olive oil, garlic, spaghetti
42	Stewed peppers	Peperonata (also spelled pepperonata) is a stew made with bell peppers, tomatoes, onions, olive oil, and garlic
43	Pasta with basil pesto 2	Pesto is one of the most popular pasta toppings in Italy made with fresh basil leaves, pine nuts, garlic, salt, Parmesan cheese, and olive oil.
44	Sweet and sour Dolphinfish	In Sicily, Mahi Mahi is called Capone. In this recipe is made with onions, capers, sugar and vinegar
45	Carrot and cabbage salad	Salad made of red cabbage, green cabbage, carrots, salt, olive oil, vinegar
46	Tomato sauce pasta	Pasta cooked in hot water with tomato
47	Caponata	An industrial typical Sicilian dish made with fried eggplant, tomato, onion, olives, celery, capers, vinegar, sugar.
48	Sparkling wine rice	Creamy dish with nearly a porridge-like consistency made with rice, Parmigiano cheese, butter, onion, spumante wine,
49	Red lentil Soup	It is a creamy red lentil soup, prepared with red lentils, onions, garlic, and sweet carrots in a tomato-based broth
50	Sausage	Roasted traditional Sicilian sausage made with Pork, Fennel, Garlic, Chilli and parsley
51	Tortellini with meat sauce	Small circles of rolled dough folded around a filling with cheese, various meats like prosciutto, mortadella with ragu sauce (onion, tomatoes, carrots, chopped meat)
52	Seasoned green olives	Green olives marinated in garlic, red wine vinegar, olive oil, chili, parsley, oregano
53	Chickpea and mussels soup	Mediterranean soup made with chickpea, mussels, garlic, olive oil, white wine, celery, carrots, rosemary
54	Wholemeal bread	Type wholemeal flour bread
55	Meatballs in tomato sauce	Meatballs are ground meat rolled into a ball, along with bread crumbs, minced onion, eggs, seasoning, cooked in tomato sauce
56	Butter arancina	Sicilian <i>arancini</i> are one of Sicily's most popular street foods. Rice balls stuffed with butter, mozzarella cheese, ham rolled in breadcrumbs and fried until crisp
57	Pasta with sardines	It is a "trademark" dish of Sicily. The principal ingredients are olive oil, onions, pasta and a finely chopped mixture of sardines and anchovy. Wild fennel, saffron, pine nuts, raisins and salt are added to flavour the dish. To finish the dish it is topped with toasted breadcrumbs.

MedDi is based on respect for territory and biodiversity, and ensures conservation and development of traditional activities and trades related to fishing and agriculture in Mediterranean communities.

Moreover, many elements are essential for the regular functioning of the human body. People health and welfare depend principally on normal supply and concentrations of metals in tissues and changes in these amounts may lead to severe problems. Consequentially, concentrations of macro and micro elements taken by food are important, in fact, minerals are present in all body tissues and fluids and their presence is necessary to maintain some essential physicochemical processes (Soetan et al., 2010; Gupta and Gupta, 2014; Dietary Reference Intakes for Energy, 2023). Mineral nutrients can be classified into major, secondary and micro or trace minerals (Orecchio et al., 2014) on their requirement by humans. Macro elements are required in quantities greater than 100 mg day<sup>-1</sup> and represent 1% or less of body weight. These include Calcium (Ca), Chloride (Cl), Magnesium (Mg), Potassium (K), Phosphor (P), Sulphur (S) and Sodium (Na). Micro elements are essential in much smaller quantities, lower than 100 mg day<sup>-1</sup> and make up less than 0,01% of body weight. Essential trace elements are Chromium (Cr), Copper (Cu), Fluorine (F), Iron (Fe), Iodine (I), Manganese (Mn), Silicon (Si) and Zinc (Zn), Ultra trace elements are a subgroup of trace minerals that are required in amounts less than 50 ng g<sup>-1</sup> in the food and are Arsenic (As), Bore (B), Molybdenum (Mo), Nickel (Ni), Selenium (Se), and Vanadium (V). Other elements, which may contribute to biological processes, but which have not been established as essential, are Barium (Ba), Bromine (Br), Cadmium (Cd), Lithium (Li), Lead (Pb), and Tin (Sn). Non-nutritive elements such as Aluminium (Al), Bismuth (Bi), Gallium (Ga), Gold (Au), Mercury (Hg) and Silver (Ag). These metals have no known function, can be detected as contaminants in food and cause toxic effects. Moreover, element deficiencies or excesses can cause major health problems. (Bailey et al., 2010).

In this context, monitoring micro and macro elements intake is necessary to evaluate the correct intake recommendations for nutrient (Bailey et al., 2010). Although element intake levels for several people are documented (Ysart et al., 1999; Radwan, Salama, 2006; Orecchio and Papuzza, 2009; Beccaloni et al., 2013; Orecchio et al., 2015) on the best of our knowledge, no information are available for the Mediterranean diet consumers.

Considering both that the Mediterranean Diet is highly recommended by nutritionists and medics and that a lot number of peoples consume Mediterranean foods, we have decided, after analytical methods validation, to evaluate the concentrations of 18 micro and macroelements (Al, As, Be, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, V and Zn) in 57 different culinary preparations used in Sicily (Italy) and critically to evaluate the results obtained in the light of the directives and of the Dietary reference values (DRVs).

These are several terms for a set of nutrient reference values that includes the *average requirement (AR)*, the *adequate intake (AI)* and the reference *intake range for macronutrients (RI)*. These values guide professionals on the amount of a nutrient needed to maintain health in an otherwise healthy individual or group of people.

*Acceptable or Reference Daily Intake (ADI)* of a chemical is the daily intake which, during an entire lifetime, appears to be without appreciable risk to the health of the consumer on the basis of all the known facts at the time of the evaluation. It is expressed in milligrams of the chemical per kilogram of body weight.

Given that in literature no data are reported about the estimated metal daily intake for peoples who follow Mediterranean diet, it is very important to know these data.

The daily intake depends both on the level of elements in meals and the amount consumed. *The tolerable daily intake (TDI)* is an evaluation of the amount of a substance in food or drinking water which is not added intentionally (e.g. contaminants) and which can be consumed over a lifetime without giving an appreciable risk to health.

From obtained data concerning elements concentrations, we calculated the human health risk using a method suggested by US-

Environmental Protection Agency (US-EPA) in order to evaluate the probability of adverse health effects in people exposed to hazardous trace metals by consumption of typical meals of Mediterranean diet. The safety risk valuation, using target hazard quotient (THQ) supported by US-EPA, designates the non-cancer risk of contaminants by the ratio between dietary intake and the reference dose (RfD) (EFSA, 2009a; EFSA, 2009b; EFSA, 2010; Khan et al., 2013).

Moreover, to discriminate the elements originating from human activities and those from natural sources and to assess the degree of anthropogenic influence, we calculated the enrichment factor (EF) (Nolting et al., 1999; Loska and Wiechuya, 2003; Orecchio et al. 2015).

## 2. Materials and methods

### 2.1. Samples

In this research, 57 samples of ready-to-eat meals representative of those of the Mediterranean diet were taken into consideration (Table 1). The dishes were, separately, prepared by the authors (all inhabitants of Sicily) using ingredients purchased in supermarkets or local markets and were cooked according to traditional recipes. Food were selected considering typical Mediterranean diet based on vegetables, fish, meat, pasta with vegetables or fish or meat, one sample of margherita pizza and one sample of cake. The characteristics and ingredients used in the preparation of the meals are shown in the Table 1. Before the analyses, samples were milled and homogenized by using a food processor (plastic-coated) and then sub-sampled for analysis. Samples were handled immediately or stored in the refrigerator at 4 °C until analysis for a period of less than 24 h.

### 2.2. Reagents and solutions

All glassware and sample containers were thoroughly washed with hot HNO<sub>3</sub> 5% solution followed by rinsing with purified water and acetone (analytical grade) respectively. These were finally kept in the oven at 110 °C overnight. To avoid sample contaminations, different glassware and pipettes were used for standards and samples.

Analytical grade chemicals were obtained from Merck and Fluka. Ultrapure water was obtained by Milli Q water purification system. Stock solutions of 1000 mg L<sup>-1</sup> (Merck, Darmstadt, Germany) and Calibration Working solutions were prepared by serial dilutions of stock in acidify solutions in Milli-Q water before each calibration.

### 2.3. Quality control and quality assurance

The detection limit (LOD) and the quantification limit (LOQ) were estimated as reported in previous papers (Antoine et al., 2012; Orecchio et al., 2014). LOD and LOQ values are reported in Table 2. The procedural blanks were obtained by subjecting ten different aliquots of a solution containing 2 mL of HNO<sub>3</sub>, 2 mL of H<sub>2</sub>O<sub>2</sub> and 100 µL of a solution containing Au 100 µg L<sup>-1</sup>, to the entire mineralization procedure. The repeatability of the whole method, was calculated as relative standard deviation (RSD %) obtained from six independent analysis of portions of the three same samples. From each meal sample, three aliquots were mineralized and each sample solution was analysed in duplicate.

Quantification limits (LOQ) (Table 2), referred to samples ready to be consumed, ranged from 0.01 mg Kg<sup>-1</sup> for cadmium and vanadium to 1.5 mg Kg<sup>-1</sup> for iron. The precision (repeatability) (Table 2) of the whole method, calculated as the relative standard deviation (RSD %), with the exception of beryllium, whose high value (19.5%) is justified by the low concentrations in the analyzed samples, ranged from 1.4% to 7.8% that are satisfactory for the purpose of this research.

### 2.4. Sample mineralization

Food samples were dried at 40 °C in an oven with air-circulation until

**Table 2**  
Quantification limits (LOQ mg Kg<sup>-1</sup>) and precision (RSD %).

	Al	As	Be	Ca	Cd	Co	Cr	Cu	Fe	Hg	K	Mg	Mn	Na	Ni	Pb	Zn	V
LOQ (mg Kg <sup>-1</sup> )	0.2	0.03	0.015	0.09	0.01	0.15	0.8	1.1	1.5	0.015	0.040	0.2	0.04	0.5	4.0	0.1	0.5	0.01
RSD (%)	1.5	7.8	19.5	1.8	2.8	1.7	1.7	1.4	1.4	5.0	1.5	2.0	1.5	1.8	1.7	1.4	1.4	3.3

it reached constant weight. A microwave oven (Milestone model MLS-1200 Mega, Milestone Laboratory Systems, Italy) with rotor of high pressure (up to 100 bar) was used for sample mineralization. About 0.5–1 g of previously dried and homogenized samples were weighted, transferred inside Teflon vessels and 2 mL of HNO<sub>3</sub> (69%) (Fluka, Milano) and 1 mL of H<sub>2</sub>O<sub>2</sub> (30%) (Fluka, Milano) were added. The instrumental conditions used for the microwave digestion were: 1 min at 250 W, 1 min at 0 W, 5 min at 250 W, 5 min at 450 W, 3 min at 600 W and 5 min at 300 W. After digestion, the clear, colourless solution was transferred into a volumetric flask and brought to volume with Milli-Q water (R N 20 MΩ cm<sup>-1</sup>) (Merck Millipore).

### 2.5. Water and organic content determination

The water content was determined by weight loss. About 5 g of an aliquot of sample was completely dried at T of 105 °C for one night in a platinum crucible. Ashes were determined by burning at 550 °C the residue of sample previously heated a 105 °C.

### 2.6. Calibration

Calibration standard solutions for each element were prepared daily by serial dilution with HNO<sub>3</sub> 2% of a multi-element standard solution containing 29 elements (Perkin Elmer Pure Plus. Atomic Analytical) (1000 ± 5 mg L<sup>-1</sup>) in 2% HNO<sub>3</sub>. The range of concentration of the calibration curves was between 0.05 and 500 µg L<sup>-1</sup> except for Hg whose range was from 0.1 µg L<sup>-1</sup> to 1 mg L<sup>-1</sup>. For ICP-MS, solutions containing <sup>89</sup>Y and <sup>187</sup>Re (50 µg L<sup>-1</sup>) were used as internal standards to compensate any signal instability or sensitivity changes during the analysis. A solution of HNO<sub>3</sub> 2% as blank was used. Were prepared three replicate of each point and calculated the linear regression. The analysis of the seven standard solutions was replicated every day. To eliminate memory effects related to the previous analysis, between two subsequent standards analysis, a 30 s washing time was established.

The determination coefficient (R<sup>2</sup>) of calibration curves ranged from 0.995 to 0.999 indicating good linearity between instrumental signals and analyte concentrations. For all investigated elements validation parameters were considered satisfactory.

### 2.7. Quantitative analysis

To quantify the analytes concentrations in the samples, various instrumental techniques were used, in particular, Al, As, Be, Ca, Cd, Co, Cr, Cu, Mn, Ni, Pb, V and Zn, were determined using a spectrometer inductively coupled plasma mass (ICP-MS), alkaline and alkaline earth metals (Na, K, Mg) and iron by atomic emission and absorption spectrophotometer (Perkin Elmer, mod. 310), mercury by an Automatic Mercury Analyzer (DMA-80, Milestone), an instrument that determines only the afore mentioned element directly on the sample.

The ICP-MS instrument used for quantification of metals was a 7700 Agilent Technologies. Scans were made by using two mass resolution settings: the low resolution (LR, 300 m/Δm) was selected for interference-free isotopes as <sup>111</sup>Cd, <sup>202</sup>Hg, <sup>208</sup>Pb and <sup>121</sup>Sb; the medium resolution (MR, 4000 m/Δm) was used for elements like <sup>27</sup>Al, <sup>59</sup>Co, <sup>52</sup>Cr, <sup>63</sup>Cu, <sup>56</sup>Fe, <sup>55</sup>Mn, <sup>60</sup>Ni and <sup>51</sup>V heavily interfered by polyatomic ion species produced by a combination of isotopes coming from plasma, matrix and reagents. The control of eventual instrumental drifts was achieved by adding internal standards (<sup>115</sup>In and <sup>45</sup>Sc at 1 ng mL<sup>-1</sup>) to the analytical solutions. To get more accurate quantification data, a

matrix-matched calibration curve on 5 data points was used.

Mercury concentration was measured directly on the food samples by using Direct for Mercury Analysis the DMA-80. This instrumental technique does not require sample preparation, gives results comparable to those obtained with Cold Vapour Atomic Absorption Spectroscopy (CV-AAS) (Orecchio and Polizzotto, 2013) and few minutes are necessary for each analysis. In the measurements carried out using the DMA-80, from 350 to 500 mg of the samples or 1.5 mL of solution samples were weighed in a quartz boat and then introduced into the instrument's combustion tube. The instrument self-seals and oxygen begins flowing over the sample at a rate of ~200 mL min<sup>-1</sup>. The solid sample is dried and then thermally decomposed by controlled heating. Gaseous combustion products are moved by an oxygen flow through a Mn<sub>3</sub>O<sub>4</sub>/CaO-based catalyst (T = 750 °C). Then sample oxidation is completed and halogens, nitrogen and sulphur oxides are trapped. Elemental mercury and other decomposition products are moved to a tube containing gold-coated sand. Here the Hg<sup>0</sup> is selectively trapped (by an amalgam with gold) whilst other products are flushed out of the system. The Hg concentration is then calculated by the software on the basis of the absorbance measured at 253.7 nm and the weight of the sample. Instrument calibration was made by using a certificate standard solution of mercury. Instrumentation has dual measuring cells for an extended analysis range of 0–600 ng mercury. The analytical range of the method was from 50 to 5000 µg kg<sup>-1</sup> when samples of 100 mg were analysed. Using a 350–500 mg sample, as in our case, a quantification limit, estimated as the blank plus 10σ (IUPAC criterion) of 15 µg kg<sup>-1</sup> was obtained with a detection limit of 6 µg kg<sup>-1</sup> estimated as the blank plus 3σ (IUPAC criterion). The accuracy of the instrument was tested by three replicate analyses of a certified reference material (Quality Consult Material QC2009 09SS1, Hg = 35.5 mg kg<sup>-1</sup>). Relative standard deviations of the three Hg analyses are about 5%. A blank was run up every 5 samples. All the reported data were blank corrected.

### 2.8. Chemometric and statistical analysis

Statistical analyses were performed using the PAST four software. To calculate significant differences the Kruskal–Wallis and Mann–Whitney U tests were conducted. P values < 0.05 were accepted as statistically significant. Correlations between investigated elements were performed by means Pearson correlation coefficient.

## 3. Results and discussion

### 3.1. Chemical analyses

#### 3.1.1. Metal analyses

In Table 3 are reported the concentration values of Al, As, Ca, Cd, Co, Cr, Cu, Fe, Hg, K, Mg, Mn, Na, Ni, Pb, Zn and V in the different food analysed samples, expressed in mg kg<sup>-1</sup> of sample. Results are expressed as the average of two analyses. By considering the differences between macro and microelements, authors have focused more attention on microelements. In details, concerning microelements, most abundant analytes detected was aluminium, followed by lead, arsenic and chromium. Aluminium also shows the greatest variability within samples. These data probably depend on the availability of aluminium in the earth's crust and from the mechanisms of adsorption and bio-magnification of the metal.



Table 3 (continued)

n°	Sample/Metal	Al	As	Be	Ca	Cd	Co	Cr	Cu	Fe	Hg	K	Mg	Mn	Na	Ni	Pb	Zn	V	H <sub>2</sub> O %	Ashes %
43	Pasta with basil pesto 2	7.6	0.0014	0.00004	72	0.0022	0.014	0.01	0.36	16	< LOQ	531	202	1.9	2569	0.10	0.008	3.1	0.0051	58.2	0.44
44	Sweet and sour Dolphinfish	5.2	0.0847	0.00009	21	0.0044	0.0055	0.05	0.57	13	0.035	3374	269	0.14	3362	0.06	0.011	4.5	0.0039	68.9	1.71
45	Carrot and cabbage salad	41	0.0014	< LOQ7	38	0.0015	0.0048	0.03	0.45	13	< LOQ	1525	98	0.64	1723	0.11	0.41	2.0	0.0038	84.0	0.63
46	Tomato sauce pasta	1.8	0.0010	< LOQ4	125	0.0057	0.0066	0.02	0.57	8.2	< LOQ	1394	179	1.8	1785	0.069	0.020	6.4	0.0020	68.0	0.22
47	Caponata	47	0.0037	0.00067	20	0.0045	0.0037	0.08	0.43	6.8	< LOQ	1869	104	0.37	3864	0.11	0.010	1.2	0.0064	80.3	1.05
48	Sparkling wine rice	12	0.0123	0.00065	12	0.0025	0.0069	0.01	0.22	11	< LOQ	6606	96	1.2	2026	0.15	0.006	1.3	0.0019	74.1	0.22
49	Red lentil Soup	84	0.0008	0.00046	31	0.0044	0.013	0.03	0.95	14	< LOQ	761	149	1.1	2308	0.29	1.1	3.0	0.0096	81.1	1.29
50	Sausage	37	0.0012	0.0016	124	0.0025	0.018	0.08	0.78	16	< LOQ	3225	241	0.77	8956	0.11	0.031	24	0.0054	39.1	2.97
51	Tortellini with meat sauce	65	0.0012	0.0017	22	0.0063	0.0087	0.02	0.80	13	< LOQ	402	103	0.81	2728	0.28	0.044	4.8	0.013	73.0	0.78
52	Seasoned green olives	30	0.0004	0.00006	39	< LOQ3	0.010	0.02	1.3	7	< LOQ	878	66	0.39	3950	0.070	0.016	0.9	0.0030	67.8	0.58
53	Chickpea and mussels soup	66	0.0600	0.00086	59	0.023	0.023	0.05	1.5	12	< LOQ	800	351	3.0	4623	0.80	0.063	6.4	0.058	71.8	0.45
54	Wholemeal bread	10	0.0034	0.00004	30	0.0033	0.014	0.35	0.89	20	< LOQ	2134	479	12	5736	0.30	0.019	16	0.0062	46.1	1.04
55	Meatballs in tomato sauce	1.2	0.0006	0.00021	62	0.0034	0.0065	0.05	0.79	14	< LOQ	1981	179	0.91	2795	0.037	0.020	29	0.0021	60.2	1.30
56	Butter arancia	89	0.0228	0.00059	54	0.0037	0.0083	0.09	1.11	11	< LOQ	542	167	1.81	4989	0.14	0.148	5.8	0.0091	54.5	1.32
57	Pasta with sardines	4.7	0.0353	0.00004	80	0.00542	0.0035	0.00	0.65	6.7	0.010	1403	255	2.70	2445	0.051	0.007	6.3	0.0063	64.6	0.71
	Mean	33	0.0076	0.001	46.0	0.00621	0.029	0.09	1.1	13	0.035	1584	195	1.6	3349	0.20	0.13	6.1	0.007	69.1	4.1
	Min	0.21	0.0004	0.000	9.8	< LOQ3	0.0021	0.0029	0.17	1.7	LOQ	17	66	0.14	855	0.018	0.0038	0.77	0.00055	16.2	1.3
	Max	163	0.0847	0.006	323	0.05320	0.50	1.02	4.6	29	0.035	6606	662	12.3	14,328	1.50	1.69	53	0.058	96.0	8.4

3.1.2. Macro element evaluation (Na, Ca, Mg, K)

3.1.2.1. Daily intake of metals (DIM). The daily intake depends both on the concentration of the elements in the meals and the amount of it consumed. Daily intake of the analytes considered in this paper was calculated using the following equation (Eq.1):

$$DIM = C_{\text{metal}} \cdot D_{\text{food intake}} \tag{1}$$

where  $C_{\text{metal}}$  and  $D_{\text{food intake}}$  represent the element concentrations in Mediterranean meal ( $\text{mg kg}^{-1}$ ) and daily intake of food, respectively. The average daily intake of food was considered  $100 \text{ mg d}^{-1}$ . Consuming the considered daily amount of food dietary intake percentages are shown in Table 4.

3.1.3. Sodium

In agreement with our expectations, sodium, compared to all other macro elements, is the one with the highest concentration because, in addition to being present in the basic ingredients, it originates from the addition of sodium chloride during the preparation of the food, according to the tastes of those who prepared the dish. The sodium content in the analysed samples ranged from  $0.85$  to  $14 \text{ g kg}^{-1}$  with an average of  $3.35 \text{ g kg}^{-1}$ . The highest concentrations were found in homemade boiled potatoes (sample n° 4)  $14.3 \text{ g kg}^{-1}$ , sausage (sample n°50)  $8.96 \text{ g kg}^{-1}$ , cereal bread (sample n°15)  $8.39 \text{ g kg}^{-1}$  and birbe breaded chicken (sample n°28)  $6.62 \text{ g kg}^{-1}$ .

The Recommended Daily Intake (RDI) for sodium is  $2300 \text{ mg day}^{-1}$  (Dietary Guidelines for Americans, 2020–2025) for healthy adults and  $1500 \text{ mg day}^{-1}$  for persons with hypertension and other health pathologies (Antoine et al., 2012; Dietary Guidelines for Americans, 2020–2025). However, people who perform intense physical activity, that generally who lose large amounts of elements through sweat, need larger amounts both macro and micro elements. Based on these considerations, and taking in to account both the consumption in a day of  $100 \text{ g}$  of above preparations that guarantees about only the 15% of the daily requirement, and looking at the data individually, we realize that  $100 \text{ g}$  of boiled potatoes (sample n° 4) would provide almost  $1.4 \text{ g}$  of sodium, a quantity lower than the tolerable daily amount ( $2.3 \text{ g}$ ), corresponding at 62% of RDI. But people who perform intense physical activity, who lose large amounts of the element through sweat, need larger amounts. In light of what has been said, on average, the consumption in a day of  $100 \text{ g}$  of aforementioned preparations guarantees about only the 15% of the daily requirement, but, looking at the data individually, we realize that  $100 \text{ g}$  of boiled potatoes (sample n° 4) would provide almost  $1.4 \text{ g}$  of sodium, a quantity lower than the tolerable daily amount ( $2.3 \text{ g}$ ), corresponding at 62% of RDI.

The sodium concentrations in the analysed samples are slightly higher than those measured in some foods for celiac (Orecchio et al., 2015) which are in the range from  $0.31$  to  $12.3 \text{ g Kg}^{-1}$  with an average of  $3.3 \text{ g Kg}^{-1}$  while in Hispanic wheat cultivars they varied between  $0.041$  and  $0.27 \text{ g kg}^{-1}$  (Rodriguez et al., 2011).

3.1.4. Potassium

Potassium content of analysed samples was found to range from  $17$  to  $6610 \text{ mg Kg}^{-1}$ . The mean concentration was  $1600 \text{ mg kg}^{-1}$ . The lowest concentrations were found in the salmon pasta (sample n°6) while the highest in rice with sparkling wine (sample n° 48). A potassium intake of  $3500 \text{ mg kg}^{-1}$  for adult men and women is considered adequate by European Food Authority (EFSA, 2016 www.efsa.europa.eu/en/efsa-journal/pub/4592), while Dietary Guidelines for American (https://www.dietaryguidelines.gov) proposes  $3400 \text{ mg d}^{-1}$  for male and  $2300 \text{ mg d}^{-1}$  for female. Considering the daily consumption of  $100 \text{ g}$  of Mediterranean food and the amount recommended by EFSA, on average, the samples considered in this paper meet the needs for about 5%.



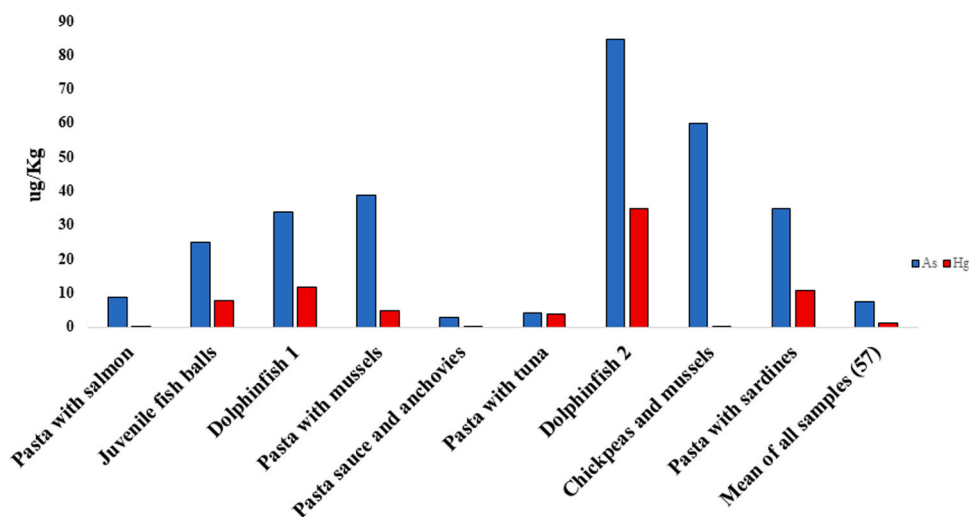


Fig. 1. As (blue) and Hg (red) concentration in some analyzed samples.

### 3.1.5. Calcium

Several researches (Stipanuk and Caudill, 2012; Stipanuk and Caudill, 2016) have clarified that correct intake of this metal keeps bones strong in old people and prevents and cures a variety of bone-related diseases, such as osteoporosis.

In the meals prepared and analyzed in this paper, calcium concentrations ranged from 9.8 mg kg<sup>-1</sup> (n° 3, roasted chicken) to 323 mg kg<sup>-1</sup> (n° 24, Juvenile fish meatballs). The average concentration of Ca is 46 mg kg<sup>-1</sup>. Adequate intake of this element for adults is 1000–1300 mg day<sup>-1</sup> depending on age and gender (National Institutes of Health (USA), 2019; Loska and Wiechuya, 2003). Considering the average concentration in the samples analyzed, a daily meals of the Mediterranean Diet contributes for about 0.46%. Tolerable daily intakes for adult male and female is established at 2000 mg d<sup>-1</sup> (National Institutes of Health (USA), 2019).

### 3.1.6. Magnesium

Magnesium concentrations in Mediterranean meal samples ranged from 66 mg Kg<sup>-1</sup> (n° 52, Seasoned green olives) to 662 mg kg<sup>-1</sup> (n°7, Portulaca salad). High concentrations of Mg were also found in two bread samples (n°15, n°54). The mean concentration found in samples was 195 mg Kg<sup>-1</sup>. The Dietary Reference Intake (RDI) for males and females is considered to be 420 mg day<sup>-1</sup> and 320 mg day<sup>-1</sup> respectively. Considering the average value of the concentrations of all the analyzed samples, males can obtain up to 0.46% of their RDA by a meal, while the contribution for females is 0.6%. Portulaca salad (sample n°7) is the food that contributes most to the daily magnesium requirement (16% for male and 21% for female).

## 3.2. Microelement evaluation (Al, As, Cd, Co, Cr, Cu, Fe, Hg, Mn, Ni, Be, Pb, Zn and V)

### 3.2.1. People health risk (THQ)

As suggested by US-Environmental Protection Agency (US-EPA), people health risk, particularly, to evaluate the probability of adverse health effects in humans exposed to certain hazardous trace element through diet. The safety risk (THQ) describes the non-cancer risk of contaminants by the ratio between exposure dose (i.e, dietary intake) and the reference dose (R<sub>FD</sub>) (US-EPA). This value was calculated using the following equation (Eq. 2):

$$\sum THQ = \sum DI/R_{FD} \quad (2)$$

where THQ is the hazard quotient, DI is the dietary daily intake in mg d<sup>-1</sup> and R<sub>FD</sub> is the reference dose in mg d<sup>-1</sup>. A THQ value < 1 indicates

that the trace hazardous metal concentration is no harmful, while a THQ value > 1 indicates that it has potential harm to the human body (US-EPA).

### 3.2.2. Aluminium

Aluminium content in the foods, prepared according to the Mediterranean tradition, ranged from 0.21 to 163 mg kg<sup>-1</sup> with an average of 33 mg kg<sup>-1</sup> and is higher than that (0.4–13 mg kg<sup>-1</sup>) determined by Greger (Greger, 1985; Lopez et al., 2002) in cereals and that of gluten-free foods (0.6–23 mg kg<sup>-1</sup>) (Orecchio et al., 2015). In our case, the highest Al concentrations were measured in stewed peppers and arancina with meat samples (n°42, n°38). Aluminium in food can arise from that which is present naturally in vegetable and animal ingredients used during meal preparation, that which contained in several food additives and that, not negligible, arising from contact with food containers, cookware, utensils and wrappings.

Considering that Al additives are contained in specific processed foods, in agreement to our expectations, the lower concentrations (< 1 mg kg<sup>-1</sup>) were found in some simple foods (boiled potatoes, boiled broad beans, rice salad, breaded chicken birbe, dolphinfish). There are emergent data on the Al toxicity, in fact, the World Health Organization in 2006 (IARC, 2006) modified the Provisional Tolerable Weekly Intake (PTWI) from 7 mg kg<sup>-1</sup> of body weight to 1 mg kg<sup>-1</sup> of body weight based on the conclusions that amounts lower than the previous PTWI may positively affect the developing nervous and reproductive systems (JECFA, 2007). In view of the tendency to accumulate in the body, Agency for Toxic Substances and Disease Registry and EFSA (ATSDR, 2008; EFSA 2008a) considered it appropriate establish a Tolerable Weekly Intake (TWI) of 1 mg Al kg<sup>-1</sup> body weight (about 10 mg d<sup>-1</sup>). While, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Scientific Committee (2018) published a provisional tolerable weekly intake (PTWI) for aluminium of 7 mg kg<sup>-1</sup> body weight (BW) per week (Stahl et al., 2017). From EFSA's considerations, it is clear that, on average, the consumption of a meal among those analysed provides about 33% of aluminium compared to the maximum tolerable value, but only in a meal (sample n° 42, stewed peppers) the metal content is higher (163%).

### 3.2.3. Arsenic

Arsenic concentrations in the analysed samples ranged from 0.004 to 0.085 mg kg<sup>-1</sup>. The highest concentrations were found in all preparations containing fish: Dolphinfish (sample n° 27) 0.034 mg kg<sup>-1</sup>, pasta and mussels (sample n°34) 0.039 mg kg<sup>-1</sup>, Dolphinfish 2 fish (sample n° 44) 0.085 mg kg<sup>-1</sup>, chickpeas and mussels (sample n°53)



**Table 5**

Health risk index of heavy metals via intake of Mediterranean meals (100 g).

	Sample	Al	As	Be	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Zn	V
n°	RfDing (mg day <sup>-1</sup> )	10	0.012	0.024	0.06	0.18	0.18	10	0.034	11	1	0.2	25	1.8
1	Chocolate cake	0.044	0.026	0.00025	0.013	0.024	0.18	0.018	0.00029	0.025	0.045	0.076	0.022	0.00063
2	Caponata	0.041	0.019	0.00045	0.0021	0.0035	0.027	0.0079	0.00029	0.015	0.0079	0.008	0.0076	0.00034
3	Roast chicken with aromas	0.99	0.037	0.0083	0.00475	0.0052	0.042	0.013	0.00029	0.0023	0.011	0.026	0.030	0.00028
4	Boiled potatoes	0.0020	0.0049	0.00007	0.060	0.0040	0.0055	0.0078	0.00029	0.00493	0.012	0.020	0.0080	0.00003
5	Baked Pasta	0.023	0.011	0.00069	0.011	0.0040	0.043	0.0104	0.00029	0.014	0.010	0.017	0.023	0.00015
6	Pasta with salmon	0.15	0.072	0.00029	0.0059	0.0027	0.022	0.012	0.00041	0.012	0.0083	0.019	0.015	0.00012
7	Porcelain salad	0.17	0.017	0.00039	0.0071	0.011	0.016	0.012	0.00029	0.033	0.015	0.016	0.018	0.00080
8	Spaghetti carbonara	0.59	0.0078	0.0012	0.0047	0.0050	0.14	0.017	0.00029	0.0099	0.024	0.04	0.016	0.00035
9	Boiled meat (muscle)	0.0074	0.0044	0.00007	0.0017	0.0020	0.020	0.016	0.00029	0.0013	0.0024	0.013	0.210	0.00010
10	Soup with green beans	0.010	0.011	0.00019	0.0037	0.0023	0.0040	0.0073	0.00029	0.019	0.0079	0.012	0.016	0.00009
11	Boiled carrots	0.11	0.0058	0.00034	0.0021	0.0018	0.0042	0.0039	0.00029	0.0048	0.0079	0.0058	0.0033	0.00018
12	Boiled zucchini	0.018	0.013	0.00019	0.0019	0.0037	0.012	0.0020	0.00029	0.0026	0.0039	0.0023	0.0031	0.00004
13	Omelette with courgette flowers	0.026	0.0042	0.00024	0.0021	0.0035	0.0074	0.014	0.00029	0.0050	0.0042	0.015	0.029	0.00029
14	White bread	1.1	0.037	0.0017	0.0073	0.022	0.17	0.029	0.00029	0.028	0.081	0.64	0.029	0.00094
15	Cereal bread	0.021	0.021	0.00030	0.023	0.011	0.098	0.024	0.00029	0.061	0.038	0.025	0.046	0.00023
16	Stewed peppers	0.42	0.019	0.0044	0.0034	0.0034	0.0059	0.011	0.00029	0.0088	0.0065	0.013	0.0073	0.00046
17	Tomato tripe	0.068	0.0095	0.00066	0.0042	0.0023	0.012	0.0081	0.00029	0.0092	0.0048	0.0060	0.042	0.00028
18	Spaghetti tomato and eggplant	0.0081	0.0068	0.00014	0.0013	0.0012	0.0028	0.0050	0.00029	0.019	0.0018	0.0019	0.013	0.00006
19	Red peppers with potatoes	0.029	0.00937	0.00031	0.0013	0.0035	0.023	0.0071	0.00029	0.012	0.0072	0.0050	0.0088	0.00010
20	Boiled broad beans	0.0047	0.011	0.00015	0.0030	0.041	0.0025	0.018	0.00029	0.019	0.031	0.0059	0.027	0.00004
21	Pizza	1.3	0.043	0.010	0.0060	0.28	0.57	0.015	0.00029	0.017	0.15	0.030	0.014	0.00045
22	Roasted aubergines	0.49	0.012	0.00056	0.055	0.019	0.025	0.016	0.00029	0.022	0.051	0.55	0.019	0.00021
23	Peperoni rossi agrodolce	0.089	0.016	0.00099	0.0035	0.0053	0.013	0.009	0.00029	0.0086	0.0047	0.0096	0.0074	0.00024
24	Juvenile fish meatballs	0.072	0.21	0.00053	0.0060	0.18	0.54	0.0070	0.023	0.0088	0.074	0.021	0.037	0.00038
25	Red Pumpkin	0.11	0.0051	0.00013	0.00048	0.0080	0.012	0.010	0.00029	0.0038	0.011	0.016	0.0091	0.00009
26	Green salad	0.019	0.0032	0.00013	0.0020	0.0013	0.0052	0.0024	0.00029	0.019	0.0039	0.0031	0.0044	0.00010
27	Dolphinfish 1	0.0097	0.28	0.00007	0.0077	0.0034	0.010	0.023	0.035	0.0020	0.0038	0.026	0.020	0.00012
28	Chicken birbe	0.0084	0.0068	0.00028	0.0055	0.0084	0.034	0.014	0.00029	0.0077	0.0097	0.042	0.017	0.00010
29	Pasta with basil pesto	0.85	0.019	0.0046	0.018	0.015	0.01	0.012	0.00029	0.015	0.051	0.041	0.014	0.00092
30	vegetable minestrone	0.026	0.0056	0.00011	0.0030	0.0077	0.0027	0.0059	0.00029	0.0073	0.012	0.0027	0.011	0.00009
31	Meat sauce	0.014	0.0064	0.00007	0.010	0.0063	0.091	0.0039	0.00029	0.0039	0.0083	0.044	0.053	0.00026
32	Pasta with zucchini	0.012	0.0069	0.00022	0.0015	0.0054	0.0066	0.0017	0.00029	0.0069	0.0035	0.0019	0.0061	0.00008
33	Grated yellow peppers	0.89	0.012	0.0047	0.0094	0.027	0.0093	0.0075	0.00029	0.013	0.022	0.0068	0.012	0.00017
34	Pasta with mussels	0.82	0.32	0.0015	0.032	0.0090	0.033	0.016	0.014	0.012	0.022	0.020	0.015	0.00278
35	Pasta sauce and anchovies	0.012	0.021	0.00007	0.0010	0.0058	0.016	0.0056	0.00029	0.028	0.0051	0.0048	0.017	0.00019
36	Rice salad	0.0067	0.15	0.00007	0.0040	0.0012	0.0091	0.0056	0.0020	0.0061	0.0038	0.0063	0.00715	0.00011
37	Pasta pesto and tomatoes	0.018	0.053	0.00007	0.0056	0.0027	0.016	0.0024	0.0038	0.012	0.0041	0.0043	0.010	0.00024
38	Arancine with meat	1.3	0.083	0.0014	0.089	0.011	0.034	0.021	0.00029	0.017	0.043	0.84	0.049	0.00098
39	Pasta with tuna	0.18	0.036	0.00055	0.0013	0.0025	0.0088	0.0025	0.012	0.013	0.0033	0.00254	0.012	0.00020
40	Chicken salad	1.1	0.018	0.0082	0.017	0.028	0.016	0.012	0.00029	0.0055	0.015	0.016	0.016	0.00020
41	Pasta with tenerumi	0.75	0.024	0.0095	0.010	0.038	0.012	0.021	0.00029	0.0066	0.015	0.022	0.011	0.00051
42	Stewed peppers	1.6	0.012	0.0030	0.018	0.013	0.031	0.046	0.00029	0.0063	0.040	0.11	0.011	0.00070
43	Pasta with basil pesto	0.076	0.012	0.00007	0.0036	0.0075	0.0080	0.0036	0.00029	0.017	0.010	0.0041	0.013	0.00028
44	Dolphinfish 2	0.052	0.71	0.00015	0.0074	0.0030	0.028	0.0057	0.10	0.0012	0.0057	0.0053	0.018	0.00022
45	Carrot and cabbage salad	0.41	0.011	0.00028	0.0024	0.0026	0.018	0.0045	0.00029	0.0058	0.011	0.20	0.0081	0.00021
46	Tomato sauce pasta	0.017	0.0085	0.00023	0.0094	0.0037	0.014	0.0057	0.00029	0.016	0.0069	0.010	0.025	0.00011
47	Caponata	0.47	0.031	0.0011	0.0076	0.0020	0.044	0.0043	0.00029	0.0034	0.011	0.0051	0.0047	0.00035
48	Rice with sparkling wine	0.12	0.10	0.0011	0.0041	0.0038	0.0040	0.0022	0.00029	0.011	0.015	0.0031	0.0053	0.00010
49	Red lentil Soup	0.84	0.0063	0.00076	0.0073	0.0072	0.016	0.0095	0.00029	0.010	0.029	0.540	0.012	0.00053
50	Sausage	0.371	0.010	0.0027	0.0041	0.010	0.044	0.0079	0.00029	0.0067	0.011	0.015	0.096	0.00030

(continued on next page)

Table 5 (continued)

Sample	Al	As	Be	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Zn	V	
n°	RfDing (mg day <sup>-1</sup> )	10	0.012	0.024	0.06	0.18	0.18	10	0.034	11	1	0.2	25	1.8
51	Tortellini with meat sauce	0.65	0.0099	0.0027	0.011	0.0048	0.0084	0.0080	0.00029	0.0073	0.028	0.022	0.019	0.00070
52	Seasoned green olives	0.30	0.0035	0.00010	0.00022	0.0056	0.010	0.013	0.00029	0.0035	0.0069	0.0082	0.0037	0.00017
53	Chickpeas and mussels	0.66	0.50	0.0014	0.038	0.012	0.031	0.015	0.00029	0.027	0.080	0.032	0.026	0.00322
54	Wholemeal bread	0.10	0.029	0.00007	0.0055	0.0076	0.19	0.0089	0.00029	0.11	0.030	0.0094	0.064	0.00034
55	Meatball	0.012	0.0049	0.00035	0.0057	0.00360	0.030	0.0079	0.00029	0.0083	0.0037	0.01002	0.11	0.00012
56	Butter arancina	0.89	0.19	0.00099	0.0061	0.00461	0.048	0.011	0.00029	0.016	0.014	0.07391	0.024	0.00051
57	Pasta with sardines	0.047	0.29	0.00007	0.0090	0.00195	0.0016	0.0065	0.031	0.024	0.0051	0.00374	0.025	0.00035

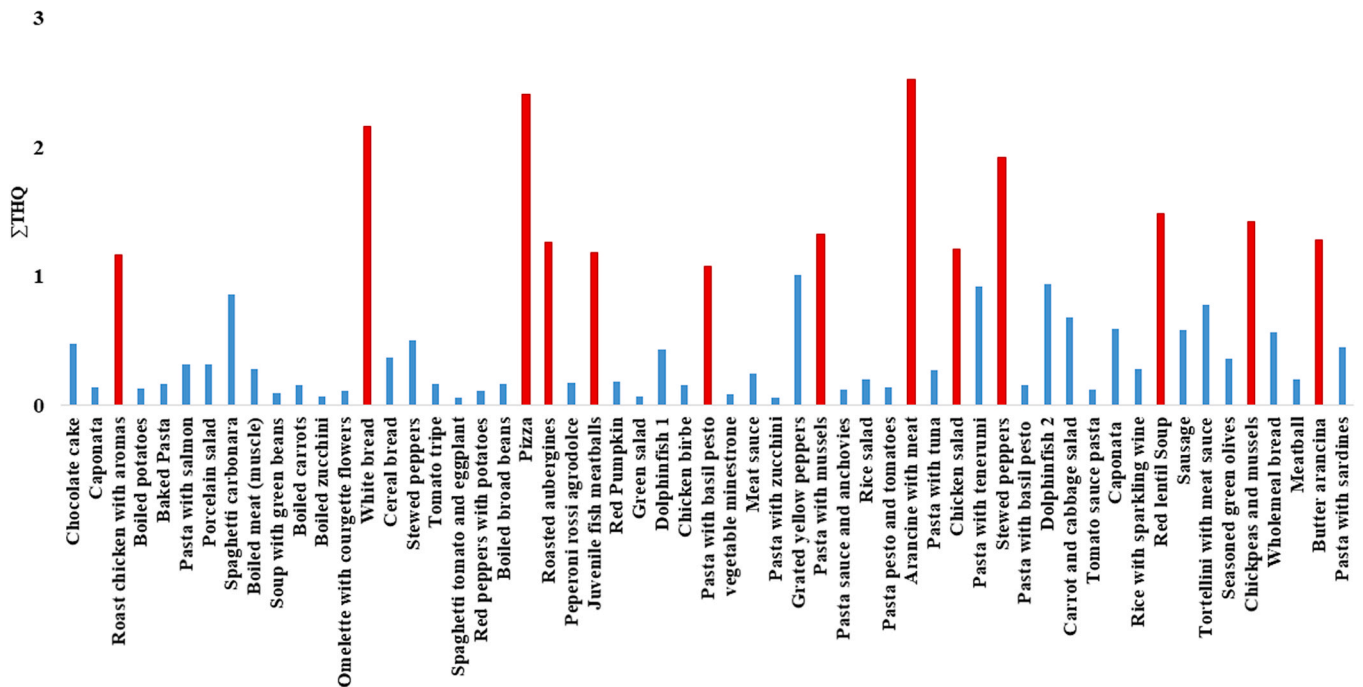


Fig. 2. Health Risk Index (THQ) for analyzed samples. In red samples whit THQ &gt; 1.

0.060 mg kg<sup>-1</sup> and pasta with sardines (sample n°57) 0.0353 mg kg<sup>-1</sup>. Our results are in good agreement with those obtained by others carried out in 19 European countries by EFSA which concluded that the daily per capita consumption of this element is in the range of 0.13–0.56 µg kg<sup>-1</sup> b.w. with higher values in specific categories, such as children under three years of age (0.50–2.66 µg kg<sup>-1</sup> b.w.) and rice and algae consumers (1 µg kg<sup>-1</sup> b.w. and 4 µg kg<sup>-1</sup> b.w.) (EFSA, 2009a). Considering that in 2009 EFSA's Panel on Contaminants in the Food Chain (CONTAM) established that the Tolerable Weekly Intake (TWI) for arsenic, corresponding to 15 µg kg<sup>-1</sup> b.w. (tolerable maximum daily intake of 2 µg kg<sup>-1</sup> b.w.) our assessment shows that this threshold is not exceeded by the consumption of 100 g of Mediterranean meals.

### 3.2.4. Cadmium

Cadmium is generally known to be a carcinogen and nephrotoxic element (Shannon et al., 2007; Huff et al., 2007), and as such is monitored and/or regulated in many food types. The WHO has also suggested a PTWI of 0.007 mg kg<sup>-1</sup> b.w. (Shannon et al., 2007).

The concentration of cadmium in the analysed meals is in the range 0.00010–0.05 mg kg<sup>-1</sup>. The highest concentrations were found in arancina with meat (sample n° 38. 0.053 mg kg<sup>-1</sup>), boiled potatoes (sample n°4. 0.036 mg kg<sup>-1</sup>) and roasted aubergines (sample n°22, 0.033 mg kg<sup>-1</sup>). Similar concentrations were found in foods containing mussels (sample n° 34, pasta with mussels 0.0190 mg kg<sup>-1</sup> and sample

n° 53, chickpeas with mussels 0.0228 mg kg<sup>-1</sup>). Even assuming the consumption of 100 g of the meal with the highest cadmium content, by an adult (60 Kg) for a week, the tolerable weekly amount, in no case would be exceeded.

### 3.2.5. Cobalt

Homogeneous data on the daily requirement of Co are not available in the literature. Some health experts acclaim that adults above the age of 18 should follow an intake limit of 1.5 µg of vitamin B12 on a daily basis and a 2.4 µg daily intake limit is recommended for adolescents. Moreover, excessive cobalt deficiency can lead to several health complications. The average concentration of Co in the analysed meals is low (0.0021 mg kg<sup>-1</sup>). Among all the samples, pizza (sample n°21) 0.50 mg kg<sup>-1</sup> and juvenile fish balls (sample n°24) 0.32 mg kg<sup>-1</sup> represent the most important sources of cobalt intake. However, on average, a normal healthy adult consuming a nutrient-rich diet ingests 5–8 µg of cobalt per day, through numerous foods (Netmeds, 2023). The tolerable upper intake levels (UI) for Co using the subchronic p-RfD of 3 µg kg<sup>-1</sup> day<sup>-1</sup>. In all the samples analysed it is always lower than the maximum tolerated concentration.

### 3.2.6. Chromium

Chromium concentration in analysed meals ranged from 0.0029 to 1.0 mg kg<sup>-1</sup>. The highest concentrations were found in pizza (sample n°

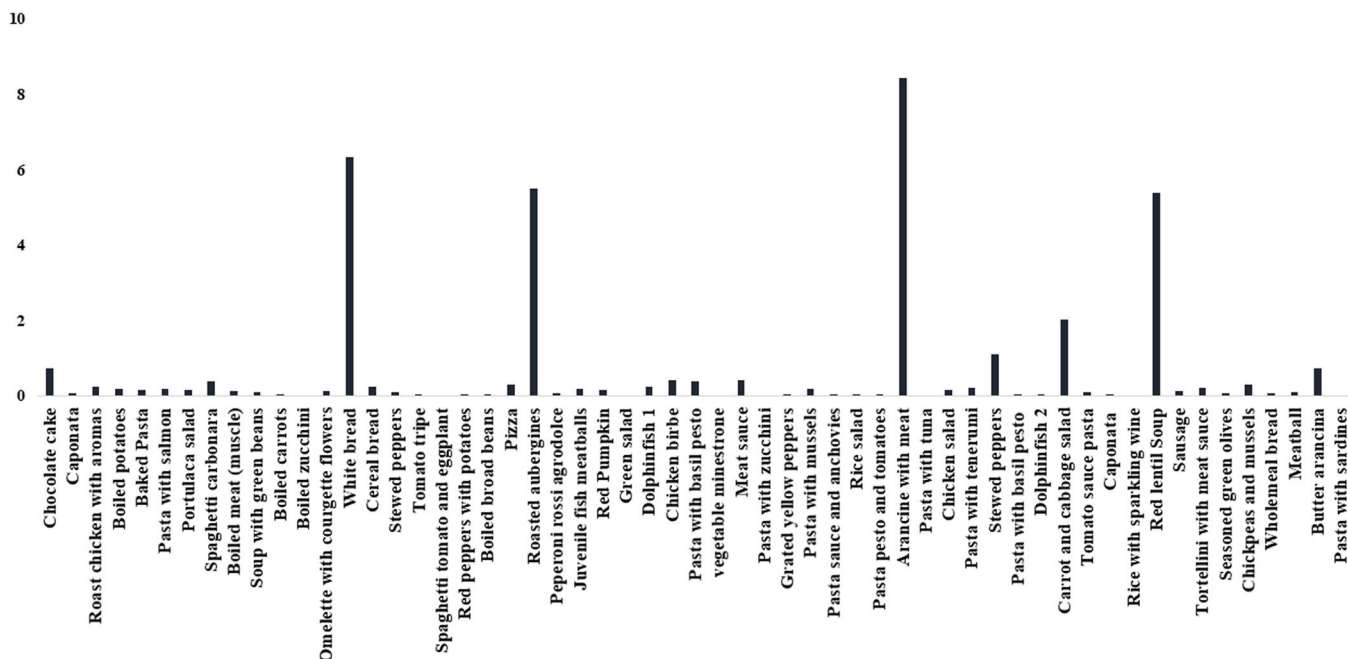


Fig. 3. Lead enrichment factor.

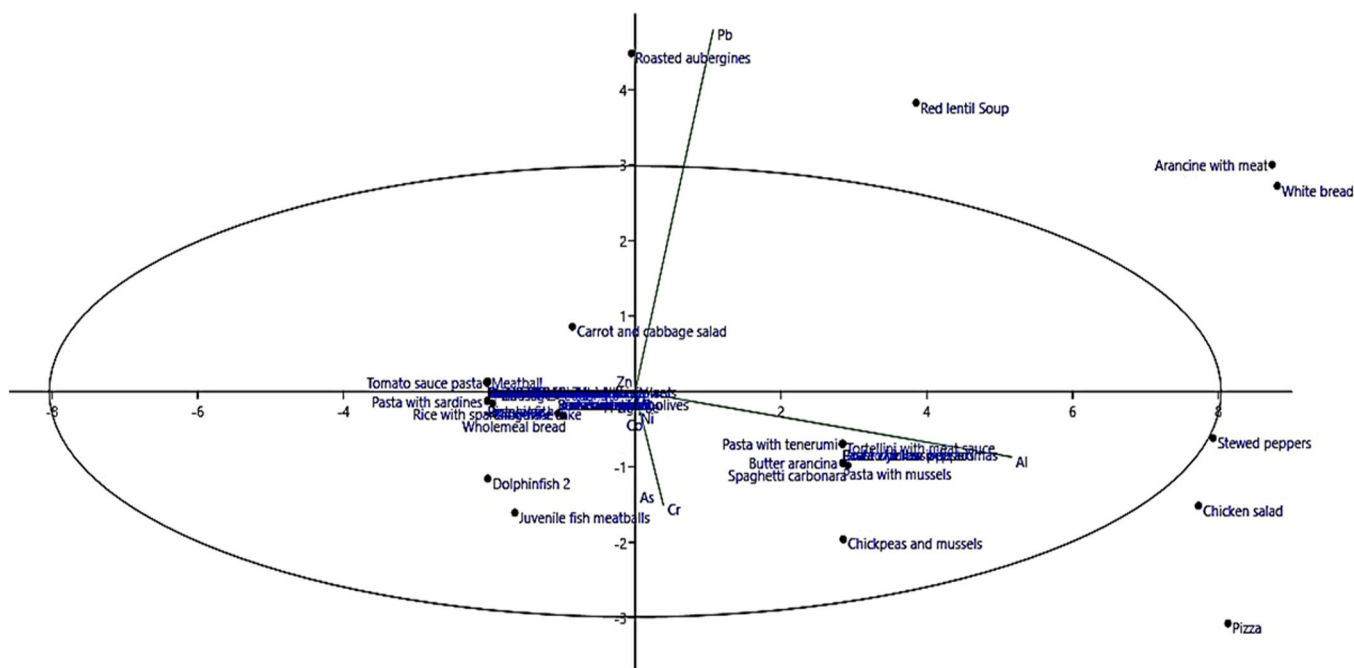


Fig. 4. Principal component analysis: scatter plot of treated values for microelement analysis in food: PCA 2 vs. PCA 1.

21)  $1.0 \text{ mg kg}^{-1}$  and in the juvenile fishes (sample n°24)  $0.97 \text{ mg kg}^{-1}$ . These results are in good agreement with the findings of other studies which asserts that the highest concentrations of this element are found in meat products ( $230 \text{ } \mu\text{g kg}^{-1}$ ), oils and fats ( $170 \text{ } \mu\text{g kg}^{-1}$ ), bread ( $150 \text{ } \mu\text{g kg}^{-1}$ ), nuts and cereals ( $140 \text{ } \mu\text{g kg}^{-1}$ ). Humans require Cr in trace amounts. Adequate intake recommendations vary by age and gender, the Recommended Daily Intake for most adult females is  $25 \text{ } \mu\text{g d}^{-1}$  and  $30 \text{ } \mu\text{g d}^{-1}$  (Noël et al., 2012). Averagely, the consumption of 100 g of the analysed meals provides between 0.5% (for men) and 0.6% (for women) of the recommended dose but, most of the samples (Pizza, Juvenile fish balls, etc.) provide quantities greater (339% and 323% respectively). The tolerable maximum daily intake ( $0.18 \text{ mg Kg}^{-1} \text{ b.w.}$ )

no exceeded in food preparations typical of the Mediterranean diet.

In another previous paper (Orecchio et al., 2014), good correlations between iron and chromium concentrations were verified ( $R^2 = 0.9384$ ) and between iron and nickel ( $R^2 = 0.9616$ ), suggesting the same origin of the three metals. Fe, Cr and Ni, in fact, are the components of stainless steel and could have been released from utensils used in food production. In the case of the foods of the Mediterranean diet that we take into consideration, there is no correlation (in term of concentrations) between the metals mentioned above. This shows that they have different origins, therefore, they are probably naturally present in the ingredients used.

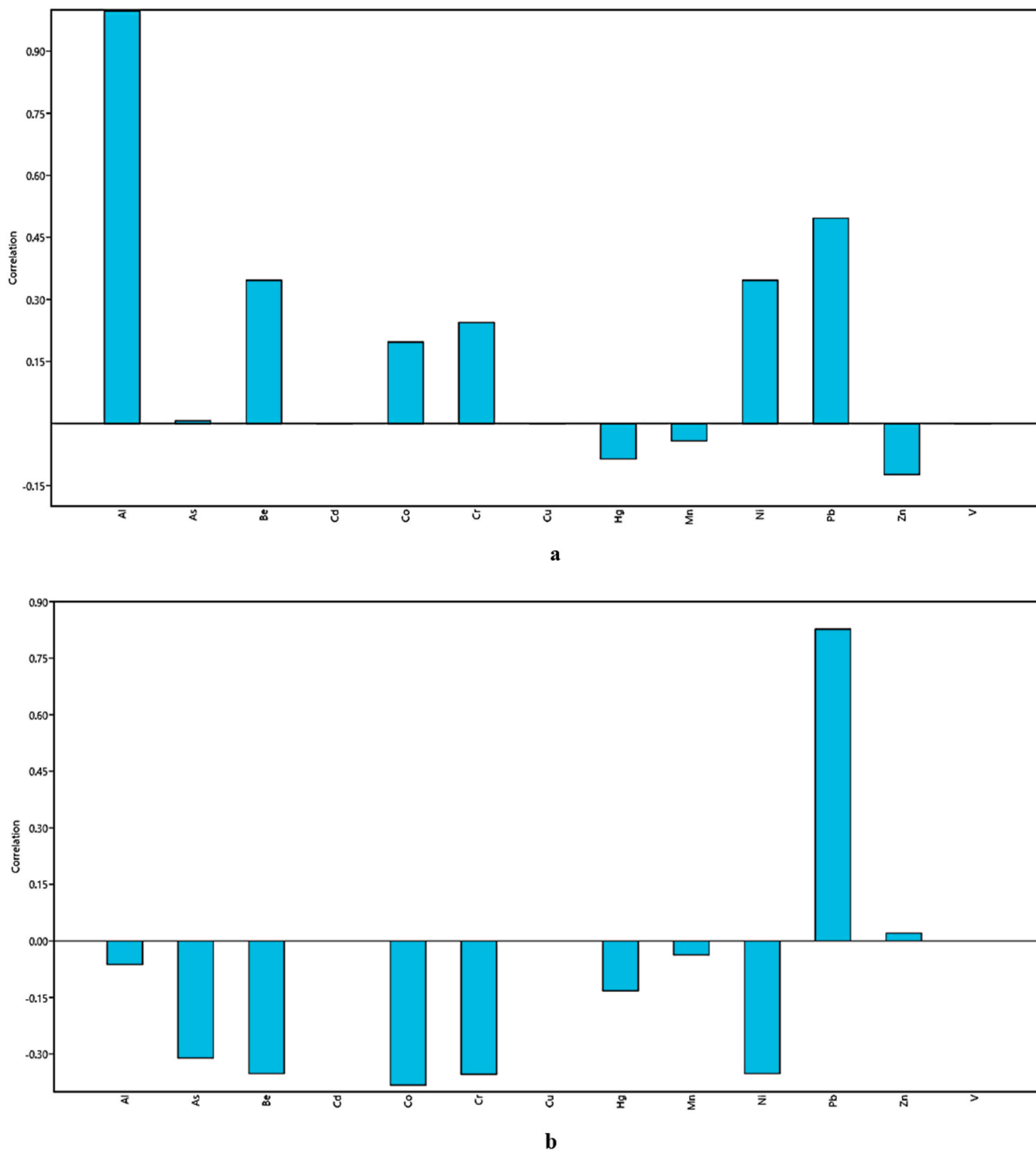


Fig. 5. a. Loading plot for PCA1. b. Loading plot for PCA2.

### 3.2.7. Copper

The copper concentration of Mediterranean diet meals samples ranged from 0.17 to 4.6 mg kg<sup>-1</sup> with an average concentration value of 1.1 mg kg<sup>-1</sup>. The highest Cu concentrations were found in stewed peppers and white bread samples (n° 42 and n° 14). These concentrations are in good agreement with what we previously determined in food samples for celiac people (Orecchio et al., 2014).

There are limited data on which human copper requirements can be based. A review of published balance studies suggests that balances can be achieved at intakes around 1.2 mg Cu daily (Commission of the

European Communities, 1993). The WHO has also suggested a dietary reference adequate intake (AI) of 0.900 mg d<sup>-1</sup>. In our case, the consumption of 100 g of the analysed meals provides from 1.9% to 51% (on average 12%) of the recommended amount. The tolerable upper intake for males and females (aged 19–70 years) of 10 mg day<sup>-1</sup> (Società Italiana di Nutrizione Umana, 2019). Also in this case, from the consumption of 100 g of meal among those taken into consideration by us, the maximum value is not exceeded.

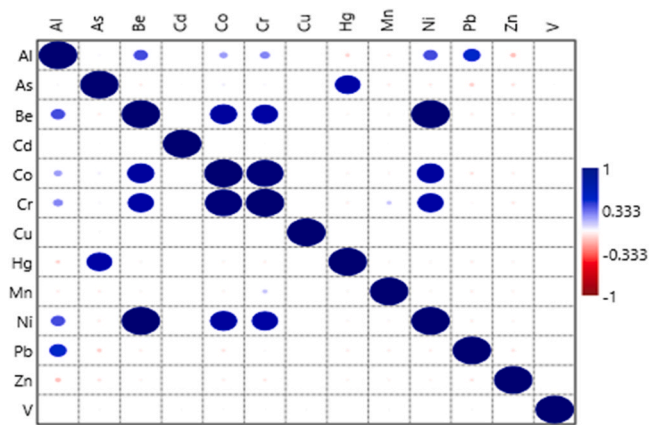


Fig. 6. Matrix correlation for metals.

### 3.2.8. Iron

In the analysed samples, iron ranged from 1.7 to 29 mg kg<sup>-1</sup>. The highest concentrations were found in the following samples: arancina with meat (sample n°38) 29 mg kg<sup>-1</sup>, pasta with mussels (sample n°34) 29 mg kg<sup>-1</sup>, in boiled meat (sample n°9) 21 mg kg<sup>-1</sup>, peppers (sample n°42) 28 mg kg<sup>-1</sup> and in chocolate cake (sample n°1) 25 mg kg<sup>-1</sup>. The daily requirement of iron (for an adult is about 7–8 mg; [Società Italiana di Nutrizione Umana, 2019](#)). On average, a portion of 100 g of the analysed meals ensures 16–18% of the requirement; Most of the meals contribute fully to the recommended dose. Tolerable daily intakes for adult male and female is established at 45 mg d<sup>-1</sup> (CFA, 2019). None of the meals considered exceeds this value.

### 3.2.9. Mercury

Mercury is a toxic metal that accumulates along the food chain and whose main source of exposure for humans is fish consumption. In meat and other foods of animal origin, its concentrations are usually low, so much so that at European level the Regulation (EC) n. 1881/2006 ([Commission Regulation EC, 1881/2006](#)) establishes the maximum concentrations of mercury only in fishery products: 1.0 mg kg<sup>-1</sup>.

Among the analysed Mediterranean meals, the maximum

concentration (0.035 mg kg<sup>-1</sup>) of Hg was found in dolphinfish 1 (sample n°44). Similarly, to what was found for arsenic, the highest concentrations were found in all preparations containing fish: pasta with sardines (sample n°57) 0.011 mg kg<sup>-1</sup>, dolphinfish fish 2 (sample n° 27) 0.012 mg kg<sup>-1</sup>, juvenile fish (sample n°24) 0.0080 mg kg<sup>-1</sup>, pasta with mussels 0.0048 (sample n°34), pasta with tuna (sample n°39) 0.0041 mg kg<sup>-1</sup>. As shown in [Fig. 1](#), arsenic and mercury concentrations in the meals containing fish products are significantly higher than the average determined by considering all meals prepared for this work.

The Hg concentrations obtained by us are in good agreement with what EFSA reports, which conducted an analysis of almost 60,000 data from 20 countries, concluding that, apart from fish, the concentration of mercury in foods is low: in the range of 0.0001–0.050 mg kg<sup>-1</sup>. with 80% of the samples below the limit of detection ([EFSA b, 2009](#)). Whereas, in 2010 the Joint FAO-WHO Expert Committee on Food Additives ([JECFA, 1986](#)) established that the tolerable weekly intake (PTWI) for inorganic mercury is 4 µg kg<sup>-1</sup> b.w. ([WHO, 2022](#)). We can conclude that the consumption of a portion or more of any of the analysed meals provides a decidedly lower amount of mercury than the tolerable one.

### 3.2.10. Manganese

Mn concentrations in the analysed samples range from 0.14 to 12 mg kg<sup>-1</sup>. Of the 57 food samples analysed, the highest levels of manganese were found in wholemeal bread n°54 (12.3 mg kg<sup>-1</sup>), followed by n°15 cereals bread (6.7 mg kg<sup>-1</sup>) and n°14 white bread (3.1 mg kg<sup>-1</sup>). The mean Mn concentration (1.6 mg kg<sup>-1</sup>) results of the same order as those reported for cereals and cereal products by Noel ([Noël et al., 2012](#)) and in gluten free food ([Orecchio et al., 2014; Orecchio et al., 2015](#)). Mean Mn concentrations in Indian, Italian, Pakistani, Thai and Vietnamese rice are in the range from 5.4 to 8.0 mg kg<sup>-1</sup>. These results agree with EFSA ([EFSA, 2006](#)). Generally, cereals, rice and nuts contain Mn concentrations from 10 to 30 mg kg<sup>-1</sup>, in fact, in the meal samples analysed, the highest concentrations were found in wholemeal bread samples (n°54). Considering that the recommended daily intake of Mn is 2.3 mg for men and 1.8 mg for women (CFA, 2019), we can say that on average the samples provide from 0.6% to 54% (meanly 6.9%) of the requirement for male and 0.8–69% (meanly 8.8%) for female. Considering that the tolerable upper diet

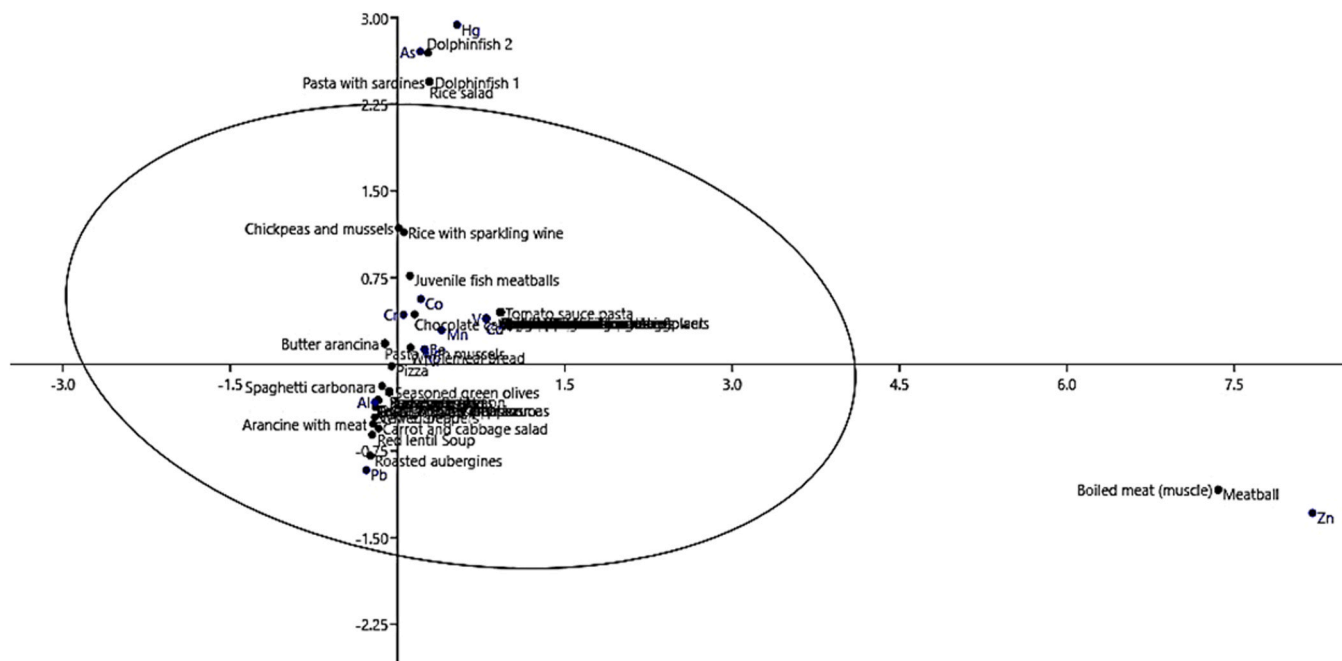


Fig. 7. Correspondence analyses carried out on analyzed samples.

intake suggested by ncbi.nlm.nih.gov is 11 mg d<sup>-1</sup> in no case is the value exceeded.

### 3.2.11. Nickel

The average concentration of Ni in our samples is 0.20 mg kg<sup>-1</sup>. According to the literature, good sources of Ni are oatmeal, dried peas, beans, nuts and chocolate (CFA, 2019); Naikoo et al., 2021). In the chocolate cake (sample n°1) the concentration of Ni results 0.45 mg kg<sup>-1</sup>. The sample with the highest concentration is pizza (sample n°21) 1.5 mg kg<sup>-1</sup>. Considering that the Ni requirement in adults does not exceed 25–35 µg day<sup>-1</sup>, on average, the consumption of 100 g of the studied meals by us ensures about 145% of the daily needs. For this element the Tolerable Upper Intake Levels (1 mg d<sup>-1</sup>) (CFA, 2019) it is never exceeded.

### 3.2.12. Beryllium

Beryllium concentration in the analysed samples is very low, in fact, the range is between 0.00004 and 0.01 mg kg<sup>-1</sup>. Samples with higher content are: roast chicken (sample n°3) 0.00497 mg kg<sup>-1</sup>, chicken salad (sample n°40) 0.0049 mg kg<sup>-1</sup>, pizza (sample n°21) 0.00629 mg kg<sup>-1</sup> and pasta with tenerumi (sample n°41) 0.0057 mg kg<sup>-1</sup>. From the consumption of a Mediterranean meal among those taken into consideration by us, in no case the Tolerable Upper Intake Levels (0.024 mg d<sup>-1</sup>) exceeded.

### 3.2.13. Lead

Lead concentrations in the analysed Mediterranean meals ranged from 0.0038 to 1.7 mg kg<sup>-1</sup>. The arancina with meat shows the highest concentration. We also found high concentrations in white bread (sample n°14) 1.3 mg kg<sup>-1</sup>, roasted eggplants (sample n° 22) 1.1 mg kg<sup>-1</sup> and red lentils (sample n°49) 1.1 mg kg<sup>-1</sup>. As regards food safety, Regulation (EC) N° 1881/2006 (EFSA, 2006) and its amendments set the maximum level of lead in meat at 0.10 mg kg<sup>-1</sup>, offal at 0.50 mg kg<sup>-1</sup>, cereals and legumes at 0.20 mg kg<sup>-1</sup> fresh weight and leafy vegetables, mushrooms at 0.10 mg kg<sup>-1</sup>. The Pb concentrations in six (white bread, roasted aubergines, arancina with meat, stewed peppers, carrot and cabbage salad and red lentil soup) of fifth-seven meals investigated by us were found to be higher than maximum levels (0.2 mg kg<sup>-1</sup> for cereals, legumes and pulses) set by EC (1881/2006) (EFSA, 2006).

Food contamination during laboratory productions or their raw material contaminations could be the main reasons for the high concentrations of this element. There is no recommended tolerable intake level as there is no evidence of thresholds for a number of critical health effects for adults (JECFA, 1986; EFSA, 2010; WHO, 2011). The mean dietary lead exposure estimates range from 0.02 to 3 µg kg<sup>-1</sup> b.w. per day. The lower value of this range (0.02 µg kg<sup>-1</sup> b.w. per day) is considerably below the exposure level of 1.2 µg kg<sup>-1</sup> b.w. per day calculated by the Committee to be associated with a population increase in systolic blood pressure of 1 mmHg. The Committee considered that any health risk that would be expected to occur at this exposure level is negligible. At the higher value of the range (3 µg kg<sup>-1</sup> b.w. d<sup>-1</sup>), a population increase of approximately 2 mmHg in systolic blood pressure would be expected to occur. An increase of this magnitude has been associated. in a large meta-analysis, with modest increases in the risks of ischaemic heart disease and cerebrovascular stroke. Considering the tolerable upper diet intake suggested by Joint FAO/WHO, in no case the values relative to our samples exceeded.

### 3.2.14. Zinc

The Zn concentration in analysed Mediterranean meals ranged from 0.77 to 53 mg kg<sup>-1</sup>. The mean zinc content was 6.1 mg kg<sup>-1</sup>. The highest concentrations are present in boiled meat (sample n°9) 53 mg kg<sup>-1</sup>, sausage (sample n°50) 24 mg kg<sup>-1</sup> and meatballs (sample n°55) 29 mg kg<sup>-1</sup>. As can be seen, the highest concentrations have been quantified in some preparations containing meat, in good agreement

with what has been established by some authors (López-Alonso et al., 2000) who state that the muscle is the site of accumulation of this metal. The Zn average concentrations obtained through this study are similar to those found in the literature (Pagliaro Avegliano et al., 2011; Orecchio et al., 2014). Considering that the recommended daily intake of zinc is 11 mg for men and 8 mg for women, the boiled meat, sausage and meatballs samples contribute for 48%, 26% and 22%.

### 3.2.15. Vanadium

Regardless of any vanadium deficiency identified in animals, for humans there is no certain evidence of being essential, therefore it has no nutritional importance (Institute of Medicine, 2001; EFSA 2004a; Filippini et al., 2020). The main source of this element for humans is the diet (Institute of Medicine, 2001). The average concentration in the analysed Mediterranean meals is 0.007 mg kg<sup>-1</sup>. In accordance with what is reported in the literature (Filippini et al., 2020), the highest concentrations were found in pasta with mussels (sample n° 34) 0.050 mg kg<sup>-1</sup> and chickpeas and mussels (sample n° 53) 0.058 mg kg<sup>-1</sup>. In vegetables, vanadium is usually present in concentrations of a few µg kg<sup>-1</sup>, in accordance with this, the samples having very low of V concentrations were boiled potatoes (sample n° 4) 0.0006 mg kg<sup>-1</sup> and boiled courgettes (sample n° 12) 0.0007 mg kg<sup>-1</sup>. From the consumption of a meal among those taken into consideration by us, in no case is the toxicity threshold exceeded (1.8 mg d<sup>-1</sup>) (CFA, 2019).

## 3.3. Daily intake of metals (DIM)

Relating to Reference Adequate Intake (RDA) for men adults (60 Kg), consuming 100 g of Mediterranean meals, dietary intakes are shown in Table 4. In particular, these supply from 3.7% to 62% of sodium, 0.05–19% of potassium, 0.1–3.2% of calcium, 1.6–16% of magnesium, 2.1–37% of iron, 0.7–48% of zinc and 1–339% of chrome. As can be seen from the data, referred to 100 g of Mediterranean meal, some of the foods prepared are lacking in macroelements. However, it must also consider that medially peoples of the Mediterranean regions consume two or three meals in the daytime (breakfast, lunch and dinner), undoubtedly, for a total of 500–1000 g. A Daily Estimated Element Intake (DEI) was calculated by following equation (Eq. 3).

$$DEI = \frac{MC \cdot DFC}{RFD} \quad (3)$$

Where MC is the average metal concentration (mg kg<sup>-1</sup>) in the food samples, DFC the daily food consumption and RFD the dietary intake or the reference dose.

## 3.4. People health risk (THQ)

Except for aluminium in stewed peppers (sample n°42), the Health Risk Index (THQ) (Table 5), for the other hazardous elements, shows no substantial risk. Considering the contributions of all the toxic elements, the sums of the single indices, for some foods (Fig. 2), are greater than one, indicates that the trace element exposure level is harmful, while a THQ < 1 would indicate that it has no potential harm to the human body (US-EPA).

## 3.5. Enrichment factor

Element enrichment factor (EF), evaluated relative to the background values (Wikipedia), was used to establish which elements were relatively enriched in the different Mediterranean food samples. Values of EF close to 1 pointed to a natural origin while those greater than 10 are considered to have a non-crustal source (Orecchio et al., 2014). In our case, the enrichment factors ranged from 2.6·10<sup>-6</sup> to 8.4, in particular, relating to lead concentrations, five samples (white bread, roasted aubergines, arancina with meat, carrot and cabbage salad and

red lentil soup) showed the highest value of EF (Fig. 3). These values reflect minimal contamination of considered samples.

### 3.6. Chemometric analyses

In order to detect other interestingly statistical information on Daily Intake of Metals (DIM) correlated to People health risk evaluation, chemometric analyses was performed. In this context, previously to chemometric investigation, experimental data were reprocessed as followed:

1. to metals that show DIM values  $\geq 1$ , a unidimensional value of 10 has been assigned;
2. to metals that show DIM value from 0.5 to 1, a dimensionless value of 5 has been assigned;
3. to metals that show DIM value from 0.1 to 0.5 a dimensionless value of 1 has been assigned;
4. to metals that show DIM value is less than 0.1 a dimensionless value of 0.01 has been assigned.

These dimensionless values were assigned by considering as high risk when DIM value is greater or equal to 1, middle risk when DIM value is comprising from 0.5 to 1 and also to considering that some people can eat about 200 g of the same food, low risk when DIM value ranged from 0.1 to 0.5 (it is unlikely that people ingest about 500 g of the same food) and no risk if DIM value is less than 0.1 (it is unreal that people ingest about 1000 g of the same food).

Interesting to note that PCA identify most of the analysed foods in a same group. Indeed, the 87.7% of analysed samples are define in the range of 95% of confidence ellipse samples, that can be drawn from the underlying Gaussian distribution (Fig. 4) and, only seven samples not are included in this confidence range. In detail, these stranger samples are Pizza, chicken salad, Stewed peppers, White bread, Arancine with meat, Red lentil soup and Roasted aubergines. As reported in Fig. 4 and Fig. 5a and b (scatterplot and loadings plot respectively), PCA1 is positively correlated (correlation  $> 30\%$ ) with Al, Be, Ni and Pb while PCA2 is positively correlated whit Pb and negatively correlated with As, Be, Co, Cr and Ni (correlation  $> 30\%$ ). This trend is in agreement whit metal analyses reported in Table 3 and underline the good predictive correlation od PCA1 and PCA2.

Moreover, statistical analysis has underline other interesting correlations between several metals considering DIM and THQ. Indeed, as reported in Fig. 6 significative correlations were found between Cr vs Co, Ni vs Be, middle correlations were found between Co vs Ni, Cr vs Ni, As vs Hg, Be vs Co and Be vs Cr, while slight correlations were found between Al vs Be, Al vs Ni, Al vs Pb.

Concerning these correlations, the most interesting would seem to Arsenic vs Mercury. Their correlation suggests a common origin. This hypothesis would seem to be confirmed by the Correspondence analysis (reported in Fig. 7) that shows mercury and arsenic metals grouped in a graphic area's characterized for fish-based foods.

## 4. Conclusion

In this paper, fifty-seven samples of Mediterranean Meals, prepared by the authors, following traditional recipes, were taken in consideration. The quantification of 17 macro and micro elements was carried out using microwave mineralization with nitric acid and oxygen peroxide, followed by three analytical techniques, in particular, Hg concentration was quantified directly on the food samples by using the DMA-80, technique does not require sample preparation, providing data similar to those obtained by Cold Vapour Atomic Absorption Spectroscopy and only few minutes for each analysis.

The obtained element concentrations indicate a considerable variability between samples, which could be due to the proportion of different ingredients used in the food compositions and to different

levels of contaminants in the production laboratories, however, all the dishes based on fish products are richer in mercury and arsenic than those based on vegetables and meat. With the exception of three samples, for aluminium, Mediterranean dishes analysed by us contain levels of hazardous metals under the limits tolerated. Considering the average concentrations of the elements found in the present study, consuming 100 g of food, it can be concluded that they are inadequate if compared with the recommended daily intake by international organisms, providing from 0.02% for Vanadium to 58% for Nickel of the RDI. However, it should be taken into account that on average the daily consumption is much greater than the 100 g previously considered because generally at least three large meals are consumed.

### CRedit authorship contribution statement

**Salvatore Barreca:** Methodology, Software, Validation, Formal analysis, Data curation. **Santino Orecchio:** Conceptualization, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Silvia Orecchio:** Methodology, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing. **Irene Abbate:** Formal analysis, Investigation. **Claudia Pellerito:** Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing - review & editing, Supervision, Project administration, Funding acquisition.

### Declaration of Competing Interest

None.

### Data Availability

No data was used for the research described in the article.

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