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# Trace elements in free-range hen eggs in the Campania region (Italy) analyzed by inductively coupled plasma mass spectrometry (ICP-MS)

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**Abstract** Eggs from hens raised on rural or domestic farms are a good indicator of environmental contamination, as the hens are in close contact with the ground and the air and can therefore accumulate heavy metals and other toxic contaminants from the environment as well as from the diet. In this paper, we report the results of the determination of 19 trace elements (As, Be, Cd, Co, Cr, Cu, Hg, Mo, Mn, Ni, Pb, Sb, Se, Sn, Sr, Tl, U, V, Zn) in 39 hen egg samples collected from domestic poultry farms in the territory dubbed the "Land of fires" in the Campania region (Italy). This area is characterized by environmental problems caused by the illegal dumping of industrial or domestic waste in fields or by roadsides. In some cases, these wastes have been burned, thereby spreading persistent contaminants into the atmosphere. The content of trace elements in whole egg samples was determined by mass spectrometer after a microwave-assisted digestion procedure. Because European legislation does not indicate maximum values of these elements in this foodstuff, the results were compared with the content of trace elements reported in literature for eggs, in particular home-produced eggs, in various countries. In some cases (Cd, Cu, Ni, Mn), the content determined in this study was in line with those reported elsewhere, in other cases (Pb, Cr), lower values were found.

**Keywords** Trace elements · Egg · ICP-MS · Hen · Free range

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## Introduction

Monitoring the levels of toxic and potentially toxic elements is one of the most important aspects of the control of environmental quality and food safety. Trace elements occur naturally in the environment and their increased presence can be attributed as a result of natural processes, such as volcanism or geochemical activities. However, human activity may increase their levels in the various environmental compartments, leading inevitably to their bioaccumulation in the food chain. Some of these elements are essential for living organisms, including humans, while others have long been known to be toxic, to the point that the European Commission (European Commission Regulation, EC No 1881 2006) has placed maximum limits on heavy metals, such as lead, cadmium, and mercury in certain foods, but not in eggs (European Commission Regulation, EC No 1881 2006). The biological effects of other trace elements are still poorly understood and available data of their toxicity are still scarce, inadequate to derive a tolerable upper intake level and therefore a maximum level in food.

Nevertheless, the European Food Safety Authority (EFSA) has issued a number of opinions recommending that the presence of some trace elements in various types of foods should be monitored and that exposure to them be reduced (EFSA 2009, EFSA 2010, EFSA 2012a, EFSA 2012b, EFSA 2014; EFSA 2014c, EFSA 2015).

In the Campania region (southern Italy), the discovery of the illegal burial of industrial waste and the illegal burning of waste from various sources—domestic, manufacturing, industrial—prompted a health alert, as some studies had correlated these phenomena with an increase of cancer cases in some areas of this region (Albanese et al. 2008; Altavista et al. 2004; Di Lorenzo et al. 2015; Fazzo et al. 2011; Martuzzi et al. 2009), and gave rise to considerable public concern.

In this area, the so-called "Land of Fires," analyses of soil and water made by the authorities have revealed the presence of some inorganic elements that could result from sources of pollution. However, comprehensive data on food production and the possible transition of these contaminants from the environment to the food chain and hence to humans are not available.

Eggs, in particular those produced by hens on rural farms or for domestic use, are an important matrix for analysis with regard to both food safety, as they are a widely consumed foodstuff, and environmental control, in fact eggs from free-range hens are considered good indicators of the contamination of the environment in which the hens live (Giannenas et al. 2009; Van Overmeire et al. 2006). In this regard, a 2012 EFSA report indicated that the levels of environmental pollutants, such as dioxins and PCBs, found in eggs from battery-reared hens were lower than those found in eggs from free-range hens that lived outdoors, and which were therefore more exposed to environmental contamination, primarily through the consumption of soil (EFSA Scientific Report—update of the monitoring of levels of dioxins and PCBs in food and feed 2012). This phenomenon has also been highlighted for trace elements in the eggs. In fact, the high contact of the chickens with the soil determines a higher concentration of certain elements such as zinc, cobalt, and toxic as lead in home-produced eggs respect to those conventional eggs produced from animals fed only with commercial feed and often without any relation to the surrounding environment (de Freitas et al. 2013).

In order to ascertain if a possible environmental contamination has had effects on the quality of the eggs thus determining an high health risk to local consumers, a study was conducted on eggs home-produced in the "Land of Fires" in the Campania Region in Southern Italy.

In this paper, we report the results of the determination of 19 trace elements; specifically, we took into consideration both elements recognized as non essential and even toxic in trace levels (lead, cadmium, mercury, arsenic), and those known as essential (copper, zinc, cobalt, selenium, molybdenum) and finally those elements which may be indicators of pollution of various origins (beryllium, uranium, manganese, thallium, strontium, tin, chromium, antimony, nickel, and vanadium).

## Materials and methods

### Samples

Thirty-nine hen egg samples were collected from small rural farms or from domestic, non-commercial producers in the area dubbed the "Land of Fires" in the Campania region in Southern Italy (Fig. 1). The eggs were from free-range hens and were sampled according to the criteria laid down in Regulation CE 836/2011 (European Commission Regulation, EC No 836/2011 of 19 2011); after collection, the samples were sent to the laboratory for chemical analyses.

### Reagents

Ultrapure Nitric acid 68 % (*v*/v), ultrapure hydrogen peroxide 30 % (*v*/v), and ultrapure water were purchased from Romil Ltd. (Cambridge, UK); standard solutions of arsenic (As), antymonium (Sb), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), copper (Cu), selenium (Se), strontium (Sr), thallium (Tl), tin (Sn), uranium (U), vanadium (V), and zinc (Zn) at 1000 mg/l were obtained from CPA Ltd. (Stara-Zagora, Bulgaria). Standard Reference Materials NIST 1549a Whole Milk Powder and NIST 1548a Typical diet were purchased by NovaChimica S.r.l. (Cinisello Balsamo, Milano, Italy). Argon gas (99.9999 %) was supplied by SAPIO S.r.l. (Monza, Italy) and anhydrous ammonia by AIR Liquide S.p.a. (Milano, Italy).

Only disposable glassware was used to avoid cross contamination.

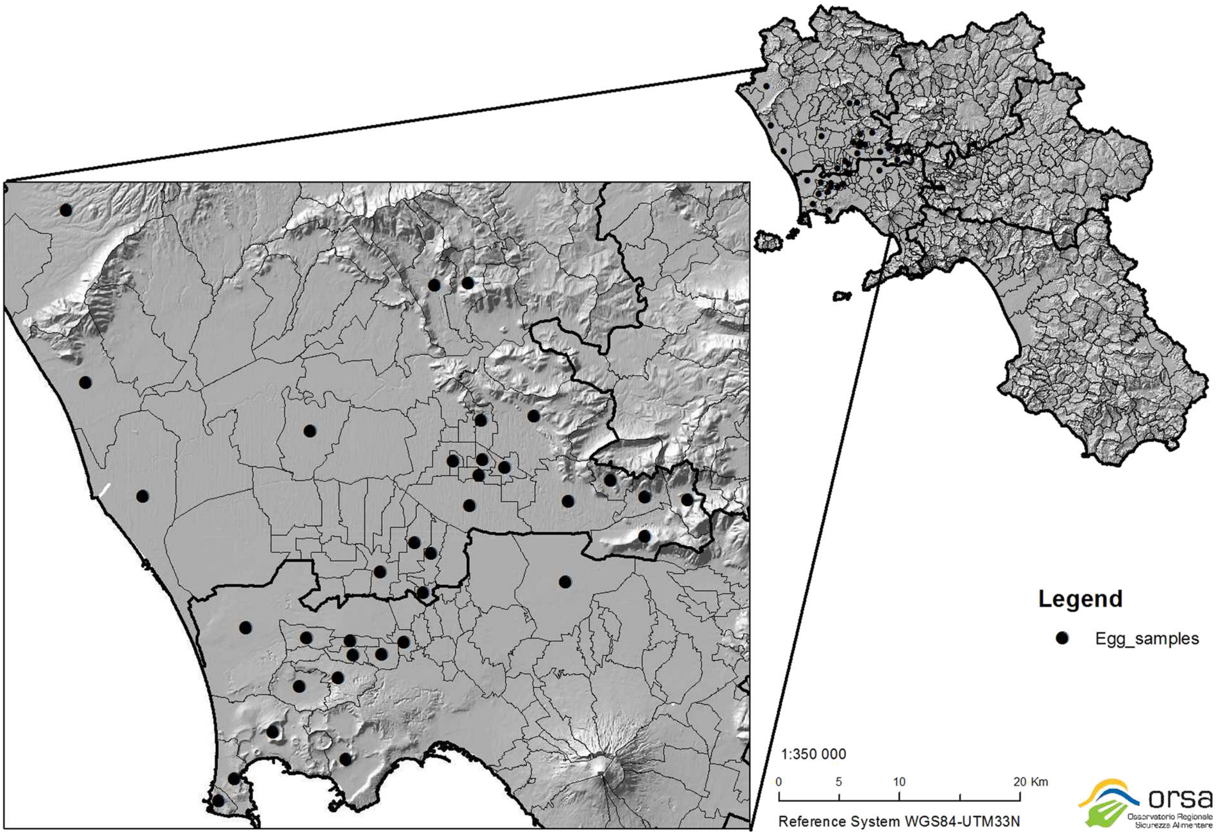


**Fig. 1** Map of region Campania (Southern Italy) showing sampling sites

Sample preparation

All egg samples were prepared according to the requirements of the standard methods UNI EN 13804:2002 (Sample preparation) and UNI EN 13805:2002 (Microwave mineralization): for each analytical sample, consisting of 12 eggs, the yolk and the egg white were homogenized together. A 2.0-g portion of each homogenized sample was weighed inside high-pressure Teflon (TFM) vessels and digested with 6 ml of nitric acid 68 % (*v*/v) and 2 ml of hydrogen peroxide 30 % (*v*/v) by microwave using a Milestone Ethos-One apparatus (FKV S.r.l., Torre Boldone, Bergamo, Italy) through the following schedule: up to 120 °C in 15 min and constant for 10 min; up to 190 °C in 15 min and constant for 20 min; cooling stage (30 min) to reach room temperature. After acid digestion, the samples were cooled to room temperature and diluted to 50 mL with ultrapure water. The same procedure was applied for blank samples (2.0 ml of ultrapure water replacing egg samples).

Analysis of trace elements

Trace elements were determined by means of inductively coupled plasma mass spectrometer (ICP-MS) (Elan DRC II, PerkinElmer, Waltham, USA); the apparatus was equipped with a concentric nebulizer (Meinhard Associates, Golden, USA), a cyclonic spray chamber (Glass Expansion, Inc., West Melbourne, Australia), and a quartz torch with a quartz injector tube (2-mm internal diameter). Analyses were performed according to the standard method UNI EN 15763:2010 opportunely modified and integrated; the instrumental parameters of the equipment used are summarized in Table 1.

Following isotopes of trace elements were considered: $^{111}$Cd, $^{202}$Hg, $^{75}$As, $^{238}$U, $^{9}$Be, $^{118}$Sn, $^{205}$Tl, $^{98}$Mo, $^{59}$Co, $^{52}$Cr, $^{51}$V, $^{60}$Ni, $^{78}$Se, $^{66}$Zn, $^{55}$Mn, $^{63}$Cu, $^{121}$Sb, $^{88}$Sr and the sum of isotopes $^{206}$Pb, $^{207}$Pb and $^{208}$Pb. To eliminate isobaric interferences, dynamics reaction cell system (DRC) was implemented, using gaseous ammonia (100 %, high purity) at 0.5 ml/min for the determination of As, Co, Cr, V, Ni, Zn, Mn, and Cu, and methane gas (99.9995 %) at 0.5 ml/min for the

**Table 1** Instrumental conditions of ICP-MS for trace elements analysis

| Parameter | Value |
|---|---|
| Radiofrequency | 1200 W |
| plasma gas (Argon) flow | 15 l/min |
| Nebulizer gas (Argon) flow | 0.97 l/min |
| Sample flush speeding | 32.0 rpm |
| Read delay | 20 s |
| Read delay and analysis speeding | 20 rpm |
| Wash | 60 s |
| Wash speeding | 36 rpm |
| Dwell time | 50 ms |
| Sweeps/reading | 20 |

determination of Se. RPq values were set at 0.75 for both reaction gases. A solution of bismuth and rhodium (approximately 200 ng/ml) added on-line was used as an internal standard.

Quantitative determination was carried out by means of the method of standard additions in the mineralized solution through the use of calibration curves at four levels of fortification; specific concentration ranges were used for each element: 0.1–0.5–2.0–10 ng/ml for As, Pb, Cd, V, Ni, Se, Co, Mo, Sn, and Cr; 0.001–0.005–0.020–0.10 ng/ml for Be, U, Hg, and Tl; 0.5–2.5–10–50 ng/ml for Cu, Sr, and Mn; 0.01–0.05–0.20–1.0 ng/ml for Sb; 4–20–80–400 ng/ml for Zn. The correlation coefficients obtained for the calibration curves were all greater than 0.995. The method was validated for the whole set of analytes under investigation by means of an *in-house* validation procedure.

The limit of quantification (LOQ) of each trace element was calculated as ten times the standard deviation of 20 mineralization procedure blanks.

### Quality assurance

The analytical method used to determine trace elements in the eggs was accredited in accordance with UNI CEI EN ISO/IEC 17025:2005. Thus, appropriate quality assurance procedures and precautions were implemented in order to ensure the reliability of the results.

The accuracy of the method was checked by using Standard Reference Materials NIST 1549a Whole Milk Powder and NIST 1548a Typical diet. For those elements (Hg, U, Be, Tl, Co, Cr, V, and Sb) not covered by a certified reference value in the two Standard Reference Materials mentioned above, spiking experiments at two concentration levels were carried out. As recovery was close to 100 % for all analytes, the results were not corrected for the recovery factor. Chemical blank determinations were made regularly, together with each batch of samples, to check for possible contamination.

### Statistical analysis

The concentrations of the trace elements in each sample were evaluated as the mean of 2 determinations, with a repeatability of less than 10 %. The results were expressed as milligram per kilogram of wet weight sample. When the content of the element was below the LOQ, the concentration was assumed to be equal to LOQ/2 (Menichini and Viviano 2004) to perform the statistical analysis.

Statistical calculation was performed by means of SPSS for Windows (Version 21, SPSS UK Ltd., Woking, Surrey, UK). One egg sample was excluded from the statistical study because the concentrations of some trace elements were obvious outliers.

## Results and discussion

The concentrations of trace elements in the egg samples are presented in Table 2, which reports mean, median, variance, standard deviation, minimum and maximum values and quartiles.

The Kolmogorov–Smirnov test (K–S test or KS test) was applied to test for normality of distribution of the data obtained: in most cases, trace elements in eggs were not normally distributed.

Descriptive statistics are presented in the form of a box plot depicting the distribution of groups of data with their quartiles (Fig. 2).

In order to detect possible correlations among the concentrations of trace elements, Pearson's chi-square test ($\chi 2$) was applied to sets of categorical data. For those elements which displayed a good correlation ($p < 0.05$), a linear regression correlation test was applied to detect any correlation between these elements. A good positive correlation was found between Mn and Cu (*R(Albanese et al.* 2007*)* = 0.640), Mn and Zn (*R(Albanese et al.* 2007*)* = 0.621), and Cu and Zn (*R(Albanese et al.* 2007*)* = 0.604); the correlation graphs between concentrations of these metals are shown in Fig. 3. As regards Zn and Cu, a

**Table 2** Values of mean, median, minimum, maximum, standard deviation and variance and LOQ for trace element content (except Be, Hg, Sb, and Sn) in hen eggs collected in Campania region

| | Median | Mean | Variance | S.D. | Range | 25th | 75th | LOQ |
|---|---|---|---|---|---|---|---|---|
| As | 0.006 | 0.007 | 0.00002 | 0.004 | 0.002–0.026 | 0.005 | 0.009 | 0.0042 |
| Cd | 0.002 | 0.003 | 0.00001 | 0.003 | 0.000–0.015 | 0.001 | 0.003 | 0.0004 |
| Co | 0.004 | 0.004 | 0.00000 | 0.002 | 0.001–0.008 | 0.003 | 0.005 | 0.0005 |
| Cr | 0.005 | 0.008 | 0.00008 | 0.009 | 0.001–0.053 | 0.003 | 0.010 | 0.0009 |
| Cu | 0.664 | 0.679 | 0.01632 | 0.128 | 0.444–1.155 | 0.605 | 0.733 | 0.0101 |
| Mn | 0.347 | 0.360 | 0.02007 | 0.142 | 0.119–0.821 | 0.251 | 0.421 | 0.042 |
| Mo | 0.114 | 0.179 | 0.03058 | 0.175 | 0.031–0.639 | 0.067 | 0.225 | 0.0015 |
| Ni | 0.058 | 0.096 | 0.00939 | 0.097 | 0.010–0.415 | 0.031 | 0.137 | 0.020 |
| Pb | 0.011 | 0.019 | 0.00076 | 0.028 | 0.001–0.146 | 0.003 | 0.021 | 0.0025 |
| Se | 0.258 | 0.313 | 0.02159 | 0.147 | 0.119–0.688 | 0.214 | 0.386 | 0.0047 |
| Sr | 0.635 | 0.745 | 0.12125 | 0.348 | 0.316–1.606 | 0.519 | 0.906 | 0.0092 |
| Tl | 0.003 | 0.010 | 0.00049 | 0.022 | 0.0002–0.113 | 0.002 | 0.006 | 0.00003 |
| U | 0.0002 | 0.0002 | 0.0000 | 0.0002 | 0.00002–0.0011 | 0.0001 | 0.0003 | 0.00002 |
| V | 0.008 | 0.007 | 0.00002 | 0.005 | 0.0003–0.015 | 0.003 | 0.012 | 0.0003 |
| Zn | 15.98 | 15.75 | 16.43 | 4.05 | 7.51–24.32 | 12.88 | 18.61 | 0.88 |

All 19 samples analyzed for Sb (49 % of the total samples) showed values <LOQ (0.0025) and all 39 samples analyzed showed content of Be < LOQ (0.0007 mg/kg), similarly for Hg and Sn almost all of the samples showed values < LOQ. All values are in parts per million (mg/kg) wet weight

significant inter-relationship has been found by other authors in home-produced eggs (de Freitas et al. 2013; Van Overmeire et al. 2006). Among all the other elements, no significant linear correlation was observed.

The content of certain elements in eggs—beryllium, antimony (only 19 samples analyzed) and tin—was below the limit of quantitation. In the case of tin, quantifiable traces were found in only two samples; similarly, mercury was detected only in 10 of the 39 samples, and at values very close to the LOQ.

Owing to the lack of previous information on the content of most of the trace elements in eggs produced in the Campania region, and in the absence of specific legislation on this issue, the results were evaluated by comparison with the limited data available in the literature.

As regards the toxic metals lead and cadmium, the egg samples showed low contamination levels: the mean values of these two metals were 0.019 and 0.003 mg/kg, respectively. With regard to lead, a

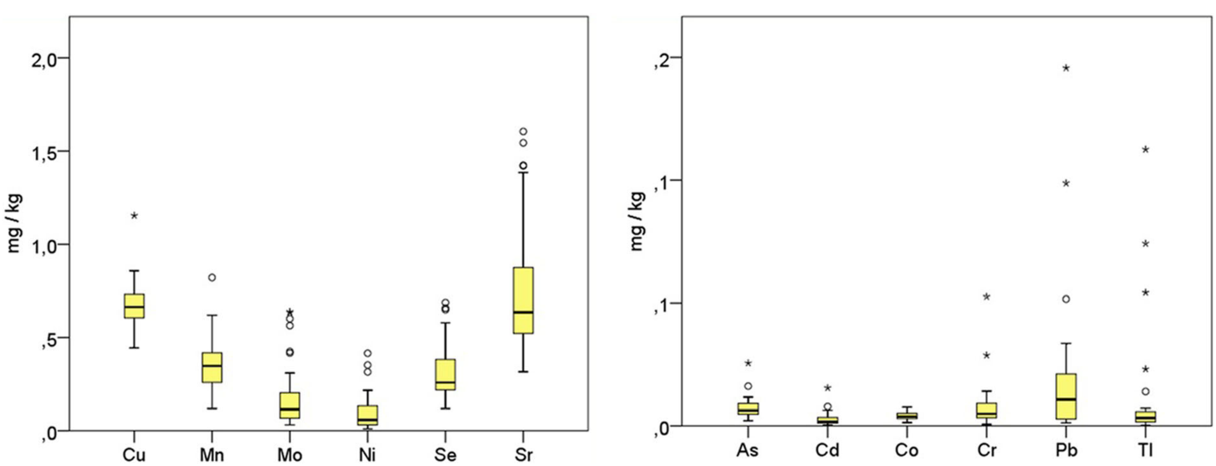


**Fig. 2** Box plot of trace elements in hen eggs

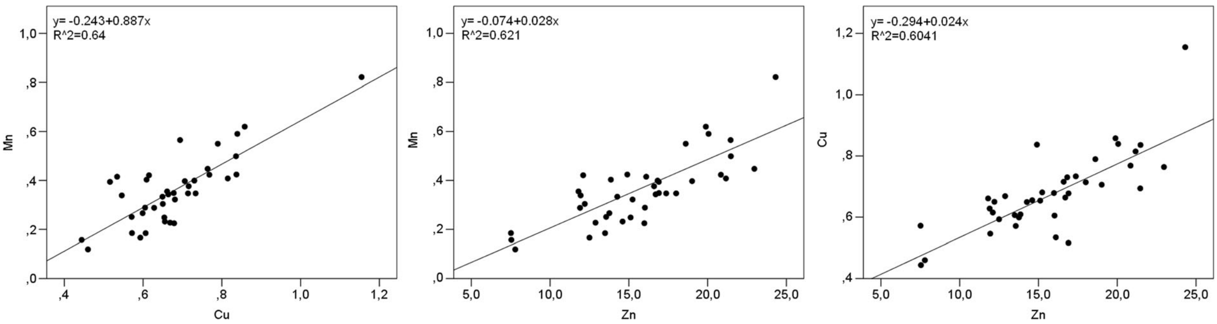


**Fig. 3** Correlation graphs between concentrations of some trace elements

significant variability in the data was also observed, as is highlighted by the amplitude of the boxplot and by the presence of some outliers (Fig. 2).

Lead and cadmium occur naturally in the environment and as pollutants released from industrial and agricultural activities. Food is the main source of human exposure to these elements, which accumulate in the body and most seriously affect the central nervous system and the kidneys. The content of lead was much lower than the concentrations found in eggs collected from private owners in Belgium (0.068 mg/kg) (Van Overmeire et al. 2006) and from local markets in India (0.489 mg/kg) (Dey and Dwivedi 2000) and Pakistan 0.59 mg/kg (Khan and Naeem 2006). Similarly, our results showed a cadmium content of 0.003 mg/kg in home-produced eggs collected in Campania, which is lower than the 0.07 mg/kg reported in eggs from India and Pakistan, but close to the values found in egg yolk in Turkey (0.003 mg/kg) (Uluozlu et al. 2009) and eggs in Finland (0.004 mg/kg) (Varo et al. 1980).

In the present study, the arsenic content of eggs showed a mean value of 0.007 mg/kg; this is relatively low, especially when compared with the data in literature (Ahmed et al. 2016; Domingo 2014; Jiang et al. 2015; de Freitas et al. 2013). However, owing to the toxicological properties of the inorganic form of this element, arsenic levels must be kept under control; indeed, the European Commission is proceeding to impose maximum limits on this element in different types of foods, starting from rice (Reg. EU 1006/2015).

Zinc concentration in eggs showed a mean concentration of 15.8 mg/kg, ranging from 7.51 to 24.32 mg/kg. In the literature, a lower value has been reported in eggs in Nigeria (6.87 mg/kg), while values as high as 24.3 mg/kg have been found in egg yolks in Turkey (Uluozlu et al. 2009) and about 20 mg/kg in eggs produced on family farms in Latvia (Vincevica-Gaile et al. 2013) and Belgium (Waegeneers et al. 2009).

The average value of copper determined in eggs from the "Land of Fires" was 0.664 mg/kg, with a range of 0.444–1.155 mg/kg; this metal, which is one of the essential elements, may originate from various industrial sources, but is mainly linked to agricultural activities, as it is contained in certain products used as pesticides. The content in our samples was also comparable to that found in eggs produced in various regions of Canada (Kirkpatrick and Coffin 1975) and very close to those observed in Belgium (0.603 mg/kg) (Van Overmeire et al. 2006), Turkey (0.8 mg/kg in yolk) (Uluozlu et al. 2009), Finland (0.70 mg/kg) (Varo et al. 1980), and Pakistan (0.78 mg/kg) (Khan and Naeem 2006), but mainly corresponds to the value of copper present in whole eggs (0.6 mg/kg), as reported in the tables of food composition prepared by INRAN (National Research Institute for Food and Nutrition, INRAN 2009).

These latter tables report selenium content of 0.058 mg/kg, which is much lower than that detected in this study in home-produced eggs in Campania (0.313 mg/kg) and very close to that found in free-range eggs in Belgium (0.273 mg/kg) (Van Overmeire et al. 2006). Selenium is of considerable importance in the diet, as its deficiency can cause damage to the thyroid and myocardium (Kashin Beck disease and Keshan disease). Indeed, the EFSA has published a review in which the need to ensure the dietary supply was highlighted. However, given the uncertainties in available data on the relationship between total selenium intake and selenoprotein concentration, they were considered insufficient to derive an average requirement for selenium in adults (EFSA 2014).

Despite an EFSA Opinion published on the biological function of chromium in regulating the metabolism of carbohydrates, lipids and proteins (European Food Safety Authority, EFSA 2014a), in a subsequent

Scientific Opinion, it was considered that there is no evidence of beneficial effects associated with chromium intake in healthy subjects, concluding that the setting of an Adequate Intake for chromium is also not appropriate (European Food Safety Authority, EFSA 2014b). From the point of view of environmental contamination, the presence of chromium is related to the use of foliar fertilizers and pesticides; however, in Campania, in the "Land of Fires," where extensive agricultural activities are carried out, the mean concentration of chromium (0.008 mg/kg) determined in eggs proved to be far lower than the concentrations found in eggs in Canada (0.06 mg/kg) (Kirkpatrick and Coffin 1975), egg yolks in Turkey (0.01 mg/kg) (Uluozlu et al. 2009), and, especially, eggs in Pakistan (0.732 mg/kg) (Khan and Naeem 2006).

The nickel mean content in the present study (0.096 mg/kg) was higher than the values found in Pakistan (0.03 mg/kg), Canada (0.04 mg/kg) but similar to that found in eggs from Turkey (0.09 mg/kg). For manganese, one of the most abundant elements in the earth crusts, the average content (0.360 mg/kg) was higher than levels found in eggs from Canada (0.29 mg/kg) (Kirkpatrick and Coffin 1975) and in free-range eggs collected in local market in London (UK) (0.277 mg/kg) (Siddiqui et al. 2011) but it was very similar to the result (0.314 mg/kg) of the analyses carried out on eggs in Belgium (Van Overmeire et al. 2006).

With regard to the average content of other trace elements, very few data are available in the scientific literature. Levels of molybdenum (0.179 mg/kg) were similar to those found in home-produced eggs from Brasil (de Freitas et al. 2013) whereas levels of vanadium (0.007 mg/kg) were lower than content in eggs obtained in different cities of Brazil (0.047 mg/kg) (de Freitas et al. 2013) and in eggs collected in the surroundings of uranium mining area in India (0.055 mg/kg) (Murad basha et al. 2013). Similarly, the values of cobalt (0.004 mg/kg) in the Campania eggs were lower than Brazilian (0.019 mg/kg) and Indian eggs (0.026 mg/kg).

Furthermore, for some of these trace elements, such as thallium (average value 0.010 mg/kg), the data showed very low variability of distribution despite the presence of many outliers (Fig 2b).

The presence of these elements in our samples may be related to a geogenic source; indeed, the highest baseline values have been recorded in the Campania region, which is characterized by the presence of an extensive volcanic area (Albanese et al. 2007). In the case of elements such as vanadium and cobalt, high levels in the soil can also result from human activities, such as the use of sludges and other fertilizers, the improper disposal of waste, combustion, and leachate. Finally, the lack of data on the presence of some trace elements, such as uranium and strontium in eggs does not allow comparisons.

Collection of these data may serve to know trace mineral composition and to establish reference values of toxic or potentially toxic metals to be used in food safety assessments of eggs produced in high environmental risk areas, such as the "Land of Fires."

In the attempt to better quantify this environmental risk, a sample of eggs that showed levels of lead, tin, nickel, and zinc up to a hundred times higher than the average values found in this study, was subjected to further investigation in order to identify possible sources of pollution in breeding hens.

## Conclusions

Hen eggs are an important component of the human diet worldwide. Various studies have detected the presence of several environmental pollutants in eggs, exposure to which might engender certain health risks for consumers. In this study, conducted on hen eggs produced in Campania (Southern Italy), we investigated the presence both of trace elements whose potential toxicity is well known and of other elements.

In conclusion, the results of the determination of trace elements revealed average contents comparable to those reported in other countries and showed a low health risk of human exposure to these elements through egg consumption. The values of these elements need further investigation in order to determine whether their presence in other foodstuff is associated with natural background values or with the spillage of waste containing metals of industrial or other origin. Because free-range rearing system has been recognized as the most susceptible to environmental variations which are not found in conventional system, it would be desirable also a study of the eggs produced in the latter rearing system in order to have a comparison on the mineral content.

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