

Arsenic Contamination of the Environment–Food Chain: A Survey on Wheat as a Test Plant To Investigate Phytoavailable Arsenic in Italian Agricultural Soils and as a Source of Inorganic Arsenic in the Diet

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Seven hundred and twenty-six samples of wheat grains from the majority of Italian agricultural areas were pooled into 141 composite samples, homogeneous with respect to geographical origin and wheat variety. The average arsenic concentration of the pooled samples was 9 ng g^{-1} , with a range of $2\text{--}55 \text{ ng g}^{-1}$ (dry weight basis). The spread of arsenic concentrations (coefficient of variation of 91%) was related to spatial variability associated with geochemical and environmental factors. Temporal variability was investigated by a 3-year longitudinal study on 7 wheat cultivars grown in 22 areas of central and northern Italy. Average year-to-year variation in arsenic levels was low, and the average of the coefficients of variation was 23%. These results show that mapping of phytoavailable arsenic in agricultural soils can be done by measuring arsenic concentration in representative samples of wheat grains. Arsenic speciation in the grain showed that As(III) and As(V) were the major As compounds, highlighting the importance of wheat as a source of inorganic arsenic in the diet.

KEYWORDS: Arsenic; speciation; wheat; food chain; phytoavailability; soil; food safety; risk assessment

INTRODUCTION

Humans are exposed to arsenic in the environment primarily through the ingestion of food and water (1–3). In recent decades, millions of people have suffered from arsenic poisoning as a result of arsenic-contaminated groundwater being used as drinking water, for crop irrigation, and in food cooking in Southeast Asia (1). Arsenic is present in water as inorganic arsenic, that is, arsenite (As(III)) and arsenate (As(V)), the most toxic forms of the element (2). Long-term ingestion of inorganic arsenic in humans is associated with skin lesions and cancers of the urinary bladder, lung, and skin (2). Developmental toxicity, neurotoxicity, cardiovascular diseases, abnormal glucose metabolism, and diabetes are other adverse effects reported to be associated with long-term ingestion of inorganic arsenic in humans, and there is also emerging evidence of negative impacts on fetal and infant development (3). In regions where arsenic contamination of water is occasional, food is the major contributor to the intake of inorganic arsenic. A recent assessment of arsenic human exposure in European countries revealed that dietary exposure to inorganic arsenic is high and should be reduced (3).

Arsenic enters the food chain through plant crops, which absorb it through their roots according to its bioavailable levels in soils. Arsenic in soil is derived from both natural and

anthropogenic sources. Background concentrations in soil range from 1 to $40 \mu\text{g g}^{-1}$, with mean values often around $5 \mu\text{g g}^{-1}$ (2,4). The nature of the soil parent material is a major factor determining the As concentration in soils, even though soils are enriched in arsenic compared with their parent rocks (4). The lowest arsenic concentrations are found in sandy soils and those derived from granites, whereas higher concentrations are found in alluvial and organic soils. However, arsenic phytotoxicity is greater in sandy soils than in other soil types because sands contain low amounts of iron and aluminum oxides and clay, which adsorb arsenic, preventing its migration in the soil solution and thus lowering its phytoavailability (5). Volcanism is an important natural source of arsenic and greatly contributes to the atmospheric flux of arsenic of natural origin. Anthropogenic sources include mining, smelting of nonferrous metals, and burning of fossil fuels. Mining activities, use of arsenical herbicides, insecticides, and wood preservatives, application of phosphate fertilizers, and irrigation with As-contaminated groundwaters are other possible contributors to the anthropogenic arsenic deposition in agricultural soils (2,4,5).

In Italy, because of the complexity of its geological history and the wide variety of substratum rock types, the natural geochemical background is highly variable. High arsenic concentrations have been detected in topsoil and subsoil in southern Campania, in eastern Lombardia, in the Roman–Neapolitan volcanic province, all along Apulia, and in central Calabria (6). Furthermore,

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Figure 1. Regions included in the study.

arsenic concentrations above $50 \mu\text{g L}^{-1}$ have been found in groundwater of several regions, including Campania, Lazio, Tuscany, Emilia-Romagna, Lombardy, Veneto, and Sardinia (7–9), which is of concern because long-term use of arsenic-contaminated water for irrigation can lead to elevated arsenic levels in soils. This geochemical background, as modified by agricultural soil management practices and impacts from other human activities, including industrial processes and other anthropogenic sources of arsenic contamination, determines the distribution of arsenic in agricultural soils. Soil surveys are carried out to investigate this distribution and obtain maps of arsenic soil concentrations (10–12). Even though these are useful tools and ensure that regulatory limits are not exceeded, they are of little use in identifying areas where accumulated arsenic in soils is actually phytoavailable and soil–plant transfer may lead to increased human and animal exposure to arsenic. Arsenic phytoaccessibility is governed by several soil factors, including pH, the contents of iron/aluminum/manganese oxides/hydroxides, redox potential, organic matter, and clay contents, factors that can differ markedly between soil types, growth seasons, and land use (5). Even soil physical conditions, such as soil compaction and irrigation, influence arsenic uptake by plants (13). As(III) is the predominant species under anaerobic conditions, and it is more soluble, mobile,

and phytotoxic than As(V) (5). As(V) is the main arsenic species in aerobic soils, and it is taken up by higher plants via the phosphate transport systems (14). Because of the chemical similarity between As(V) and phosphate, application of phosphate causes the desorption of As(V) from soil colloids and increases its concentration in the soil solution, enhancing arsenic phytoavailability (15). Arsenic taken up by plants is mainly retained in the root system, with limited translocation to the above-ground plant organs. However, the most toxic inorganic forms usually predominate in food and feed crops and present a food safety problem.

Chemical extractions have been used to estimate the plant-available fraction of trace elements in soils (16). However, these procedures are time-consuming and cannot give a reliable measure of element bioavailability because they are not universal but largely soil specific (16). In a previous study, we accomplished biogeochemical mapping of selenium in Italian agricultural soils by measuring the selenium concentration of representative samples of wheat grains (17). Wheat is the main cereal cultivated in Italy, and it is grown all over the country: bread wheat (*Triticum aestivum* L.) in the northern and central regions and durum wheat (*Triticum durum* Desf.) in the central and southern regions. Like selenium, arsenic is a nonessential element for plants, and the

concentrations in plant tissues largely reflect environmental levels. Furthermore, wheat grain is largely used as food and feed, and it is itself an important source of food contaminants in human and animal diets.

The aims of the present study were to investigate the geographical distribution of bioavailable arsenic in Italian agricultural soils using wheat as a test plant and to examine arsenic speciation in grain to assess the role of wheat as a source of inorganic arsenic in the diet.

EXPERIMENTAL PROCEDURES

Sampling of Wheat. *Geographical Variability.* Several hundred wheat grain samples ($n = 726$) from the majority of Italian agricultural areas and belonging to the most cultivated wheat varieties were collected at harvest. Representative samples were obtained by applying pre-established criteria for the collection of individual samples and their grouping into composite samples. The number of individual samples was proportional to the surface under cultivation in each region, and only the prevailing wheat varieties in each growing area were selected. Individual samples were pooled into 141 composite samples, each weighing 1 kg and homogeneous with respect to geographical origin (province) and wheat variety. Analytical determinations were performed on composite samples. Overall, 80 composite samples belonging to 8 varieties of *T. aestivum* from northern and central regions varieties and 61 composite samples belonging to 6 varieties of *T. durum* from central and southern regions were surveyed. The 14 selected wheat cultivars (see the Supporting Information, Table SI-1) altogether accounted for the majority of the Italian wheat production.

Four of the 20 Italian regions were not included in the sampling plan, being mainly mountainous territories yielding marginal wheat production (Figure 1). In the regions surveyed, the large majority of the provinces were assayed. The areas of the Italian provinces range from about 21,000 ha to about 752,000 ha (mean = 293,000 ha). All of the statistics in this paper were calculated considering the provinces (i.e., administrative districts) as reference areas.

Temporal Variability. A longitudinal study concerning seven cultivars of *T. aestivum* grown in various locations of central and northern Italy was performed with the aim to monitor year-to-year variation in arsenic levels on a short-term basis (3 years). Altogether, 22 distinct composite samples were collected each year at harvest in representative fields located in the selected sites for a total of 66 samples.

Sample Preparation. Wheat grains were cleaned of dust and other extraneous materials in a wheat cleaner (Labofix 90, MCKMaschinenbau, Germany), and damaged kernels were removed manually. The grains were ground for subsampling by an RM100 agate mortar mill (Retsch GmbH & Co., Haan, Germany). About 0.35 g of the resulting flour was placed in digestion vessels with 3 mL of ultrapure concentrated HNO_3 (Carlo Erba Reagenti, Milan, Italy) and 1 mL of ultrapure H_2O_2 (Merck, Darmstadt, Germany) for dissolution by closed vessel microwave digestion using a Milestone MLS-1200 Mega MW oven (FKV, Bergamo, Italy; irradiation program is given in the Supporting Information, Table SI-2). Samples were cooled to room temperature, transferred to polypropylene disposable test tubes and, after the addition of rhodium ($10 \mu\text{g L}^{-1}$) as internal standard, diluted to a mass of 50.000 g with ultrapure deionized water obtained from a Milli-Q system (Millipore, Molsheim, France).

Separate dry weight (dw) determinations were performed on test aliquots of 10 g for each composite sample. The average moisture contents were 10.9 and 9.9% for *T. aestivum* and *T. durum*, respectively (grand mean = 10.3%). The analytical results are expressed on a dw basis.

Total Arsenic Determination. Analytical determinations were performed by inductively coupled plasma mass spectrometry (ICP-MS) using a quadrupole Sciex 6000 spectrometer (Perkin-Elmer, Norwalk, CT). Details can be found in the Supporting Information, Table SI-3. Working standard solutions were prepared daily by diluting a 1 g of As L^{-1} stock solution (Merck) with ultrapure 1% (v/v) HNO_3 . Concentrations were determined using five-point calibrations with external standards. The detection limit of the method, calculated as 3 times the baseline noise (3σ) of 10 digestion blanks, was 0.7 ng of As g^{-1} of dw.

Analytical Quality Control. Each sample was digested and analyzed in duplicate. Digestion blanks were analyzed together with samples

belonging to the same analytical batch, and arsenic concentrations in samples were calculated after blank subtraction. Standards were run regularly after 20 sample measurements. Duplicate analyses of selected samples were carried out on different days, and results agreed within 10%.

Two wheat-based certified reference materials (CRMs), namely, SRM 1567a Wheat flour (NIST, Gaithersburg, MD) and BCR CRM 189 Wholemeal flour (IRMM, Geel, Belgium), were included in the analytical batches. The indicative values for arsenic in the two CRMs are 0.006 and $0.018 \mu\text{g g}^{-1}$, respectively. Found values and standard deviations were equal to $0.0056 \pm 0.0006 \mu\text{g g}^{-1}$ ($n = 6$) and $0.017 \pm 0.001 \mu\text{g g}^{-1}$ ($n = 6$) for the two CRMs, respectively. Spike recoveries in selected samples were $94 \pm 3\%$ (spike concentration $1 \mu\text{g of As L}^{-1}$, $n = 6$).

Determination of Arsenic Species. For arsenic speciation analysis, 10 randomly selected samples (0.5 g) were extracted in duplicate using a 1:1 (v/v) methanol/water mixture (5 mL) and sonication at 50 °C for 2 h (Ultrasonic Cleaner, VWR International, Milan, Italy). The above extraction procedure was repeated five times plus an overnight step performed under mechanical agitation. After each extraction, samples were centrifuged (10 min, 8000 rpm, 4 °C). The supernatants were combined, concentrated to 5 mL using a Zymark TurboVap II Concentrator Workstation (FKV, Bergamo, Italy), and filtered through a $0.22 \mu\text{m}$ filter. The extraction efficiency was 81% on average as calculated from the sum of the arsenic species detected by HPLC-ICP-MS.

Stock solutions of 1 mg mL^{-1} , expressed as As, were prepared by dissolving in ultrapure deionized water adequate amounts of arsenic trioxide (As(III)), disodium hydrogen arsenate heptahydrate (As(V)), dimethylarsinic acid (DMA) (all from Fluka, Dorset, U.K.), and methylarsonic acid (MMA) (from Tri Chemical Laboratories Inc., Yamanashi, Japan). Standard stock solutions were stored at 4 °C, and the exact concentrations were ascertained by ICP-MS analysis. The purity of the standards was checked by HPLC-ICP-MS, and limited species interconversion was found only in the case of the inorganic species. Quantitative results were corrected accordingly.

Arsenic species were determined by means of HPLC-ICP-MS using a metal-free HPLC system (Perkin-Elmer series 200) with an anion exchange PRP-X100 column ($250 \times 4.6 \text{ mm}$, $5 \mu\text{m}$) (Hamilton Co., Reno, NV) connected via PEEK capillary tubing to the nebulizer of an Elan DRC II ICP mass spectrometer (Perkin-Elmer-Sciex). A PC^3 Peltier-cooled quartz cyclonic spray chamber (Elemental Scientific Inc., Omaha, NE) set at 2 °C was used for sample introduction. Separations were carried out in isocratic mode using 10 mM $\text{NH}_4\text{H}_2\text{PO}_4$ and 10 mM NH_4NO_3 at pH 5 as mobile phase and an injection volume of $100 \mu\text{L}$. Arsenic species were identified by retention time matching with the standard substances spiked to the sample extracts. Quantitative calculations were based on peak areas using the method of standard additions. Interference assessment was performed at m/z 77 ($^{40}\text{Ar}^{37}\text{Cl}$). The detection limits (3σ criterion, 10 method blanks) for As(III), As(V), DMA, and MMA were 0.23, 0.71, 0.24, and 0.22 ng of As g^{-1} of dw, respectively.

Statistics. Arsenic concentrations were not normally distributed (Shapiro-Wilk W test). Frequency distributions were positively skewed for both *T. aestivum* and *T. durum*. The differences in arsenic concentrations between wheat species, wheat varieties, and wheat from different regions were tested by both Kruskal–Wallis test on untransformed data and parametric ANOVA after logarithmic transformation ($p = 0.05$).

For the map of bioavailable arsenic in agricultural soils, the arithmetic mean of arsenic concentrations in wheat was calculated for each province (m_p) and the deciles of m_p distribution were determined. The first two intervals, that is, those having the deciles D_1 and D_2 as cut points, were combined, and the same was done for D_3 – D_4 , D_5 – D_6 , and D_7 – D_8 . The resulting six intervals (cut points D_2 , D_4 , D_6 , D_8 , D_9) were associated with different colors. Each province was assigned to one of the six intervals on the basis of the m_p value.

Statistical analysis was performed by means of SPSS (version 17.0, SPSS, Inc.).

RESULTS AND DISCUSSION

Distribution of Bioavailable Arsenic in Italian Agricultural Soils. The arsenic concentrations in *T. aestivum* ($n = 80$) and *T. durum* ($n = 61$) were 8 ± 8 and $9 \pm 8 \text{ ng g}^{-1}$ of dw, respectively. The two values were not statistically different, nor was any significant

difference detected among the varieties within each species. Therefore, the two sets of data were combined. Arsenic is a nonessential element for plants, and the concentrations in plant tissues generally reflect environmental levels. In wheat, arsenic taken up by the roots is transferred to the shoots to a limited extent. Greenhouse pot experiments of wheat exposed to 2 mg of As L⁻¹ in irrigation water resulted in root levels of ~2–3 μg g⁻¹, whereas arsenic in shoots averaged ~0.65 μg g⁻¹ (18). In the same experiments the S^{shoot}/R_{oot} transfer factors were ~0.7–0.8 for wheat with ~0.05 μg of As g⁻¹ in the grain and decreased with greater root arsenic uptake (18), in accordance with earlier results of other authors (19). Levels in grain are lower than those in shoots being G^{grain}/S_{hoot} transfer factors < 1, typically ~0.2–0.3 (18, 20). Grain arsenic can be used as a probe for the

Table 1. Arsenic Concentrations in Representative Wheat Samples from Italian Regions

| origin | n ^a | As ^b (ng g ⁻¹ of dw) |
|----------------|----------------|--|
| Veneto | 10 | 13.6 (5.4) |
| Piedmont | 7 | 11.8 (15.4) |
| Lombardy | 11 | 8.5 (2.9) |
| Emilia-Romagna | 30 | 6.4 (2.3) |
| northern Italy | 58 | 10.1 (3.2) |
| Toscana | 8 | 6.3 (3.8) |
| Marche | 13 | 5.5 (2.4) |
| Umbria | 4 | 4.6 (3.0) |
| Lazio | 6 | 16.4 (19.9) |
| central Italy | 31 | 8.2 (5.5) |
| Apulia | 9 | 14.8 (10.9) |
| Sardinia | 6 | 9.7 (2.2) |
| Campania | 6 | 7.9 (4.7) |
| Basilicata | 8 | 7.9 (2.2) |
| Sicily | 11 | 7.4 (2.8) |
| Calabria | 3 | 6.7 (4.2) |
| Abruzzo | 4 | 6.4 (1.8) |
| Molise | 5 | 6.0 (0.9) |
| southern Italy | 52 | 8.3 (2.9) |

^aNumber of composite samples. ^bAverage arsenic concentration (standard deviation in parentheses).

phytoavailable arsenic in soil if once absorbed by the roots and transported to the shoots the element is transferred to the grain, that is, no control on shoot-to-grain export exists. Down-regulation of shoot-to-grain export may occur in wheat, but it was not detected at shoot arsenic concentrations up to 1.6–2.1 μg g⁻¹, corresponding to grain arsenic concentrations of 0.21–0.50 μg g⁻¹ (20). In another study, evidence of decreased arsenic translocation from shoot to grain was found in the presence of phosphate fertilization for wheat with >0.05 μg of As g⁻¹ in the grain (18). In the present study the highest arsenic concentration in the grain was 0.055 μg g⁻¹, and thus a linear relationship between bioavailable arsenic in soil and arsenic in grain can be assumed.

The arsenic data (*n* = 141) grouped by region and wide geographical area (northern, central and southern Italy) are summarized in **Table 1**. Bioavailable arsenic differed significantly among regions (*p* < 0.01), and there was a 4-fold difference between the highest and lowest levels. Higher values were recorded in Piedmont and Veneto, that is, on the north side of the Po river valley, in Lazio, and in Apulia. This geographical variability in phytoavailable arsenic was further investigated by studying the variability of arsenic concentrations in samples from each province. The coefficient of variation (CV) of arsenic concentrations, which was equal to 91% for all samples and 54% within regions (average CV for data grouped on a regional basis), was 39% within provinces (average CV for data grouped on a provincial basis). It is apparent that the spread of the arsenic concentrations measured was related to spatial variability of bioavailable arsenic in soils associated with geochemical and environmental factors. However, this apparent spatial variability could be biased by the temporal variability of arsenic levels, that is, by year-to-year fluctuations of arsenic concentrations in wheat at each location. A longitudinal study on wheat grown at 22 locations of central and northern Italy was performed with the aim to monitor year-to-year variation in arsenic levels during three consecutive years. The average arsenic concentrations at all locations were 7.1 ± 4.5, 6.8 ± 2.8, and 6.0 ± 2.0 ng g⁻¹ in the three years (CV = 9%). No significant difference was detected among the different wheat varieties investigated. Apart from a few anomalous values (e.g., sample 3, year 1, **Figure 2**), small year-to-year variations were detected and the average CV was 23%

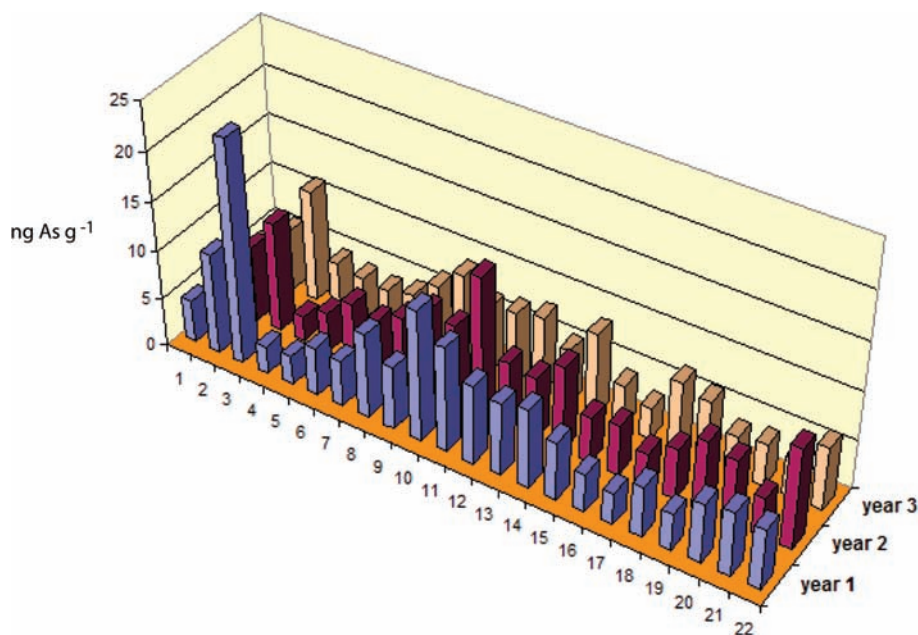


Figure 2. Temporal variations of arsenic content in wheat grain collected at 22 different locations for 3 consecutive years.

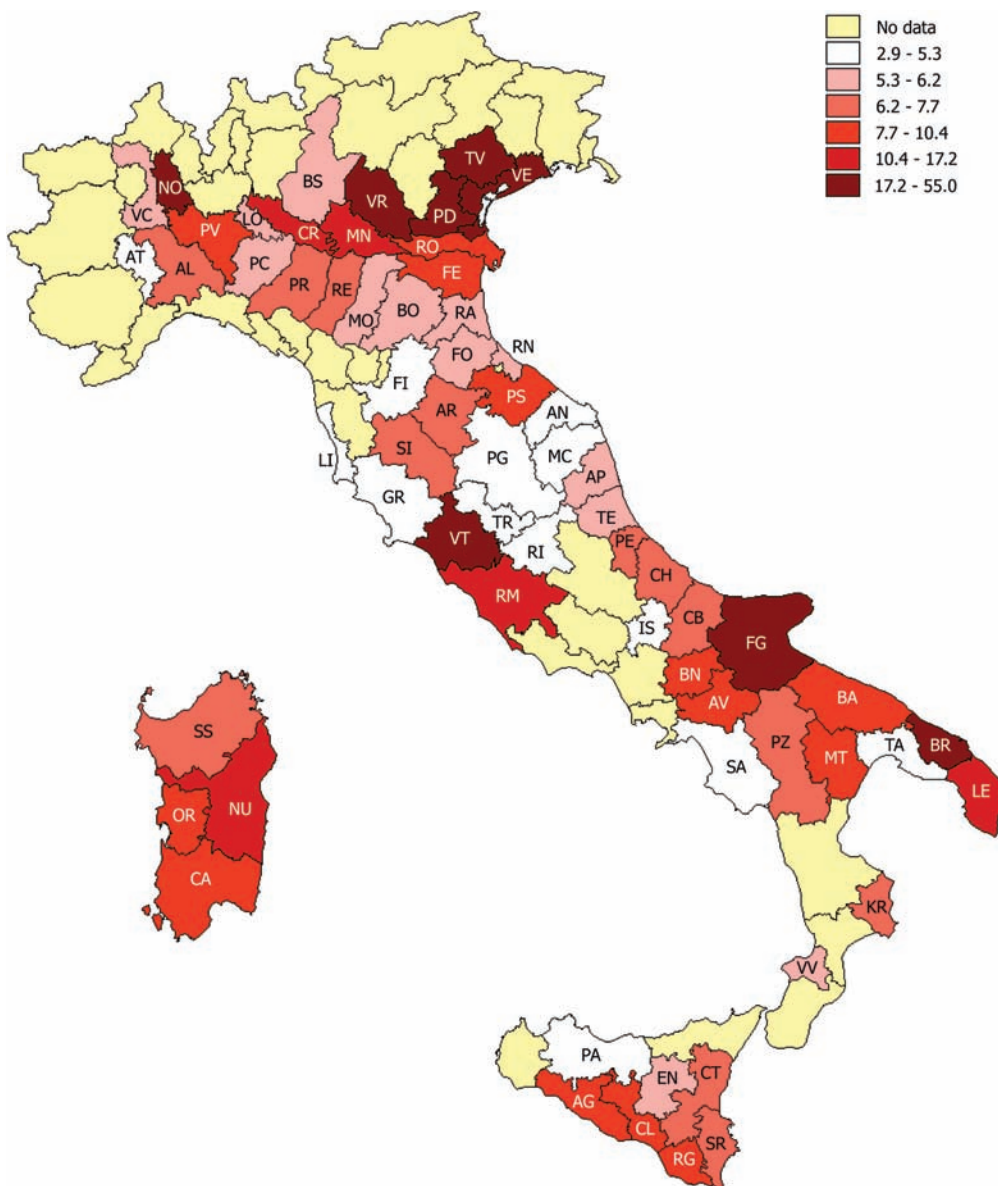


Figure 3. Distribution of bioavailable arsenic in the agricultural soils of the Italian provinces (values are arsenic concentrations in wheat expressed in ng g^{-1} of dw). AG, Agrigento; AL, Alessandria; AN, Ancona; AP, Ascoli-Piceno; AR, Arezzo; AT, Asti; AV, Avellino; BA, Bari; BN, Benevento; BO, Bologna; BR, Brindisi; BS, Brescia; CA, Cagliari; CB, Campobasso; CH, Chieti; CL, Caltanissetta; CR, Cremona; CT, Catania; EN, Enna; FE, Ferrara; FG, Foggia; FO, Forlì; FI, Firenze; GR, Grosseto; IS, Isernia; KR, Crotona; LE, Lecce; LI, Livorno; MC, Macerata; MN, Mantova; MO, Modena; MT, Matera; NO, Novara; NU, Nuoro; OR, Oristano; PD, Padova; PA, Palermo; PE, Pescara; PG, Perugia; PR, Parma; PS, Pesaro; PV, Pavia; PC, Piacenza; PZ, Potenza; RA, Ravenna; RG, Ragusa; RE, Reggio Emilia; RI, Rieti; RN, Rimini; RM, Roma; RO, Rovigo; SA, Salerno; SI, Siena; SR, Siracusa; SS, Sassari; TA, Taranto; TR, Terni; TE, Teramo; TV, Treviso; VC, Vercelli; VE, Venezia; VR, Verona; VT, Viterbo; VV, Vibo Valentia.

(all data included). It must be emphasized that because this 3-year study was not carried out in experimental fields under controlled conditions, the observed variability was likely due not only to yearly fluctuations of climatic and other variables but also to possible changes in agricultural practices at the local level. On the basis of these data, it appears that information gained through the analysis of representative samples from a single harvest can be used to obtain a reasonably reliable picture of arsenic status in soils and crops grown therein because the variability of the measured arsenic levels largely reflects the actual geographical variability of bioavailable arsenic in soil with a minor bias due to the specific sampling year.

The distribution of bioavailable arsenic in the agricultural soils of the Italian provinces is presented in **Figure 3**. As explained under Statistics, the map was designed in such a way that the areas

enriched in bioavailable arsenic were clearly discriminated from those with moderate and low levels by associating the two darker colors in the arsenic abundance scale to the upper two deciles of the distribution of arsenic values (10.4 and 17.2 ng g^{-1} of dw). As shown in **Figure 3**, 13 provinces are in the lowest interval of bioavailable As concentrations (white color), whereas 26 provinces lie in the two next intervals. In all of these areas, bioavailable As is on average lower than the national average.

Areas with Agricultural Soils Enriched in Bioavailable Arsenic. Eight provinces fall within the highest interval of bioavailable arsenic concentrations, and four of them, namely, Verona, Padova, Treviso, and Venezia, are located in Veneto (northeastern Italy). These four provinces, along with those of Mantova and Cremona located in Lombardy, are part of an arsenic-rich sector of the northern Po River Plain. This plain is the widest Italian soil

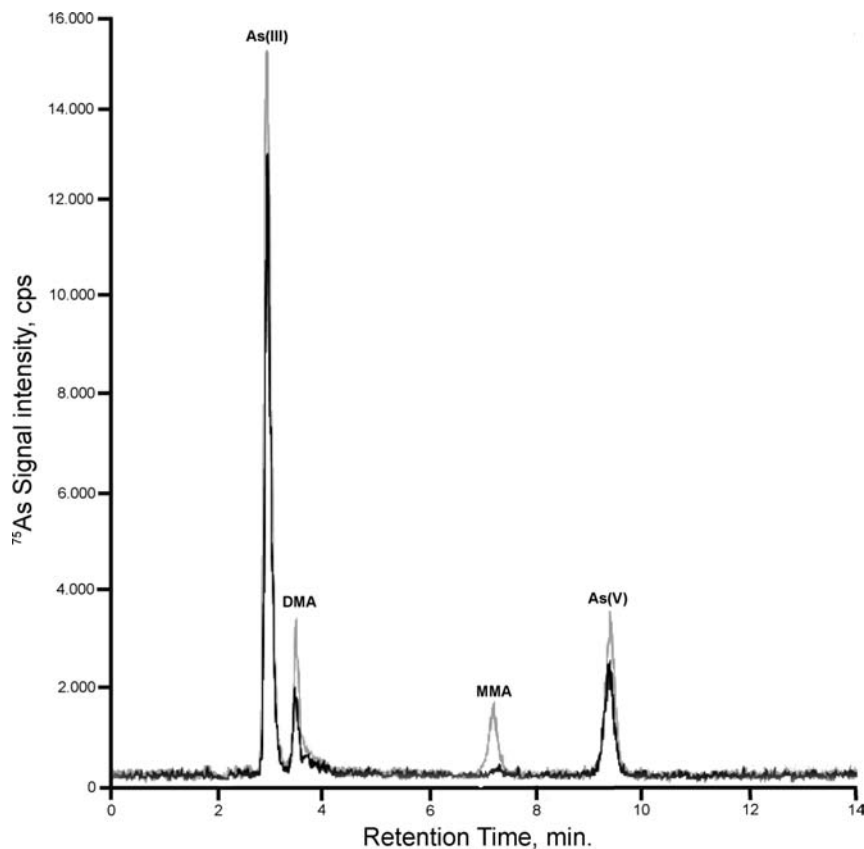


Figure 4. HPLC-ICP-MS chromatogram of an extract of wheat grain containing 25 ng of As g⁻¹. The black line corresponds to the sample and the gray line to the sample spiked with a mixture of authentic standards.

region, the Padano–Veneta Plain, characterized by soils developed on Quaternary alluvial and glacio-fluvial deposits and a temperate–suboceanic climate (21).

Arsenic contamination of Veneto groundwaters has been known for some years, with concentrations > 10 μg L⁻¹ and up to 480 μg L⁻¹ in 10–20% of wells (8). Wheat is a winter crop and usually does not need irrigation. However, use of contaminated groundwater may contribute to arsenic enrichment of soils where wheat is grown due to crop rotation. Soil arsenic levels higher than regulatory limits (20 mg kg⁻¹) have been recently reported in the Brenta and Adige Plains, which extend over the above-named provinces of Veneto (12). A detailed study of the soils in the Brenta Plain found a pedo-geochemical background value for arsenic of 36 mg kg⁻¹ (11). This relatively high concentration was mainly due to lithogenic enrichment, with a spatial distribution that was related to the nature and age of the soil parent material. Nevertheless, topsoil As contamination due to anthropogenic inputs was present at some locations and was estimated to involve about 6% of the area (11). The present study shows that soil arsenic in southern Veneto is not only moderately high, as found in the above-mentioned survey, but also phytoavailable, which has implications in terms of transfer along the food chain.

Another area in the Po River Plain with high bioavailable arsenic in soil is the province of Novara, in Piedmont (northwestern Italy). Wheat arsenic levels are much higher in this area than in the arsenic-rich district of Veneto (46.4 vs 17.3 ng g⁻¹ of dw). High lithogenic arsenic has been recently measured in soils of the Novara province (Renzo Barberis, personal communication). Arsenic enrichment of crops grown therein has not been reported previously, and it is shown here for the first time.

The province of Viterbo (Lazio, Central Italy) shows the highest level of arsenic in wheat (55.0 ng g⁻¹ of dw). The

neighboring province of Rome is also slightly enriched in bioavailable arsenic. In these areas, coastal plains and included hills are formed by Quaternary alluvial deposits with inclusions of Tertiary metamorphic rocks with a Mediterranean subcontinental to continental climate. In the hinterland, hills on effusive volcanic rocks dominate with a Mediterranean oceanic to suboceanic climate. High arsenic levels have been detected in groundwaters of the Lazio region, with concentrations > 10 μg L⁻¹ and up to 195 μg L⁻¹ in 62% of volcanic waters from the Viterbo area (northern Lazio) (9). In the same area, high arsenic levels have been found in stream sediments and floodplain sediments, and anomalously high levels have been detected in stream waters (6).

Areas with high bioavailable arsenic within the Apulia region (southeastern Italy) include the provinces of Foggia and Brindisi in the highest decile (arsenic in wheat = 21.8 and 17.2 ng g⁻¹ of dw, respectively) and that of Lecce in the following decile. In these provinces, soils developed on Quaternary alluvial and marine deposits (mainly clay and loam, with hollow limestone), along with Mesozoic limestone, marl, and residual deposits in southern Apulia (Brindisi and Lecce). The climate is Mediterranean subtropical to subcontinental. Surface soils from agricultural areas in nearby Brindisi and Lecce were found to be moderately enriched with As by an earlier study, which showed that 54% of samples contained 20–30 mg of As kg⁻¹ (10). High arsenic levels were also detected in stream sediments and floodplain sediments, especially in the Brindisi area (6).

It is worth noting that the areas found to be high in bioavailable arsenic in the present study differed in most cases from the arsenic-rich areas identified by the existing environmental geochemical maps (6). Notably, this happens for the provinces of Novara and those in the Veneto region (northern Italy). This may be due in part to the sampling strategy used in geochemical

mapping, which follows the criteria of the FOREGS program aimed at providing large-scale, baseline data for total elements (22). The present study focused on agricultural soils and employed higher density sampling. More importantly, it dealt with the bioavailable fraction, as determined by using wheat arsenic, and not with total soil arsenic. Because arsenic bioavailability is controlled by factors other than the arsenic content of the soil parent rocks, geochemical maps are unsuitable for assessing the risk for humans and animals associated with soil–plant transfer of arsenic.

The present results show that measuring arsenic concentration in representative samples of wheat grains is a useful approach for the characterization of geographical variations of bioavailable arsenic in agricultural soils at the nationwide level, and it allows identification of areas where arsenic entry in the food chain is greater. In those areas, surveys with higher spatial resolution should be carried out to characterize the exact distributions of phytoavailable arsenic at the local level. By combining this with information on the cultivated crops and their use (e.g., animal versus human consumption), a useful tool for risk assessment would be provided. Suitable risk management measures could be taken as a result, for instance, avoiding the cultivation of crop types that reach high arsenic levels in edible tissues.

Total Arsenic and Arsenic Species in Italian Wheat. The average arsenic content of the 141 composite samples analyzed in this study was $0.009 \mu\text{g g}^{-1}$ of dw, with a median and geometric mean of $0.007 \mu\text{g g}^{-1}$ of dw. Individual arsenic levels spanned a concentration range of about 1 order of magnitude, that is, $0.002\text{--}0.055 \mu\text{g g}^{-1}$ of dw. This relatively narrow range is due to the fact that composite samples were analyzed, which made the detection of extreme values unlikely.

In terms of average concentrations, the levels found in the present study are comparable with the lowest values reported so far for wheat grain, that is, $0.003\text{--}0.013 \mu\text{g g}^{-1}$ dw in field experiments conducted in the United Kingdom and various European countries (13, 23) and $0.005 \mu\text{g g}^{-1}$ of dw (range = $0.001\text{--}0.010 \mu\text{g g}^{-1}$) in an Indian survey (24). Higher levels have been found in larger scale surveys or at contaminated areas, that is, $0.069 \mu\text{g g}^{-1}$ (range = $0.041\text{--}0.101 \mu\text{g g}^{-1}$) at an arsenic-rich site in France (23), $0.053 \mu\text{g g}^{-1}$ of dw (range = $0.006\text{--}0.335 \mu\text{g g}^{-1}$) in The Netherlands (25), $0.03 \mu\text{g g}^{-1}$ of dw (range = $0.01\text{--}0.21 \mu\text{g g}^{-1}$) and $0.07 \mu\text{g g}^{-1}$ of dw (range = $0.01\text{--}0.50 \mu\text{g g}^{-1}$) in Scotland and England, respectively (20), $0.055 \mu\text{g g}^{-1}$ of dw (range = $0.020\text{--}0.091 \mu\text{g g}^{-1}$) in Serbia (26), and $0.061 \mu\text{g g}^{-1}$ of fw in Pakistan (27). Arsenic concentrations from $0.36 \mu\text{g g}^{-1}$ (28) to $0.71\text{--}0.74 \mu\text{g g}^{-1}$ of dw (29) were reported in wheat grown in arsenic-affected areas in West Bengal (India). Greenhouse experiments of wheat grown with irrigation water containing $2 \text{ mg of As L}^{-1}$ and with $50 \mu\text{g of As g}^{-1}$ spiked soil found grain levels of 0.65 and $0.69\text{--}0.75 \mu\text{g g}^{-1}$ of dw, respectively (18, 20).

To study arsenic speciation at the low total arsenic levels found in this study, a method allowing ultrasensitive detection of arsenic species was employed. Figure 4 shows a typical HPLC-ICP-MS chromatogram obtained, where a major peak corresponding to As(III) appears followed by As(V) as the next abundant species. The two inorganic species were the main compounds present in the extracts of the samples submitted to speciation analysis and accounted for 95% (range = $86\text{--}100\%$) of the sum of the detected species on average (Table 2). DMA was a very minor species in some samples, whereas MMA was detected only at trace level in one sample. A recent study showed that As(III) was the main arsenic species (86%) in the xylem sap from As(III)-treated wheat (29). Interestingly, As(III) accounted for 45% of the arsenic in the xylem sap of As(V)-treated wheat, suggesting As(V) reduction in roots prior to efflux and xylem loading (30).

Table 2. Arsenic Speciation in Grain from 10 Wheat Cultivars Grown in Italy (Mean \pm SD, $n = 2$)

| sample | As concentration (ng g^{-1} of dw) | | | | i-As ^a (%) | recovery ^b (%) |
|--------|--|-------|-----------------|------|-----------------------|---------------------------|
| | As(III) | As(V) | DMA | MMA | | |
| 1 | 15.67 | 6.07 | 0.94 | nd | 96 | 76 |
| 2 | 13.76 | 5.67 | nd | nd | 100 | 69 |
| 3 | 10.44 | 5.03 | 2.36 | nd | 87 | 77 |
| 4 | 8.97 | 6.24 | nd | nd | 100 | 82 |
| 5 | 7.36 | 3.90 | 0.87 | nd | 93 | 84 |
| 6 | 6.67 | 4.64 | tr ^c | 0.41 | 97 | 89 |
| 7 | 6.94 | 3.49 | tr | nd | 100 | 80 |
| 8 | 4.95 | 4.25 | 0.84 | nd | 92 | 77 |
| 9 | 5.50 | 3.99 | nd | nd | 100 | 68 |
| 10 | 2.37 | 3.03 | 0.87 | nd | 86 | 104 |

^a Inorganic arsenic, sum of As(III) and As(V) divided by sum of As species. ^b Sum of As species determined by HPLC-ICP-MS divided by total As concentration by acid digestion and ICP-MS determination. ^c Concentration near the detection limit.

The same authors subsequently studied arsenic speciation in wheat grain and found only inorganic arsenic to be present in the extracts, with As(III) accounting for 64–90% of the chromatographed arsenic (23). However, owing to the low detection power of their method, they could determine arsenic species only in contaminated samples containing ca. $0.050\text{--}0.100 \mu\text{g}$ of As g^{-1} . The present study shows that As(III), followed by As(V), predominates also in wheat with normal arsenic levels ($< 0.030 \mu\text{g}$ of As g^{-1}). Methylated species could be detected at low concentrations in some samples in the present study, likely due to the lower detection limits of the HPLC-ICP-MS method we used compared to that of Zhao et al. (23). Nevertheless, they accounted for $\leq 5\%$ of the chromatographed arsenic on average.

Implications for Human Intake of Inorganic Arsenic. In terms of food safety it must be emphasized that even though arsenic levels in wheat are about 1 order of magnitude lower than in rice (3, 20), almost all of the arsenic appears to be present as inorganic arsenic. Thus, the percentage of the total arsenic in the inorganic form is higher than that (70%) assumed by EFSA as the most likely scenario in their recent assessment of arsenic in food (3). This points to the importance of wheat as a potential major contributor to the dietary inorganic arsenic intake of those populations with a predominantly wheat-based diet due to high consumption of wheat-derived products. Even though milling reduces the arsenic content of grains (23, 27, 31), it is to be noted that further processing almost invariably involves addition of water for dough preparation, which is most likely to cause an increase of the inorganic arsenic content of the final ready-to-eat food. Limited and conflicting information is available in the literature. Schoof et al. (32) found 28% of the arsenic in flour to be in inorganic form, compared to 96% found in semolina by Matos Reyes et al. (33). Diaz et al. (34) found 72–87% of total arsenic to be present as inorganic species in bread from an arsenic endemic area where water is likely to significantly contribute to the inorganic arsenic content of processed food. Clearly, further research on arsenic speciation in wheat-derived products and on the influence of processing on arsenic species is required. However, on the basis of the results of the present study and considering that the water used in processing and cooking contains inorganic arsenic only, it is sensible to assume that 100% of arsenic is in inorganic form in wheat processed products for human consumption. Average European adults consume about 0.26 kg of cereals and cereal products per day, with wheat-based products being the main component (35, 36). Using a daily consumption of wheat products of 0.17 kg and the lower bound and upper bound arsenic concentrations reported by EFSA for

cereal and cereal products, recalculated excluding rice grains and rice-based products (i.e., 51.9 and 70.8 μg of As kg^{-1} , respectively), the daily intake of inorganic As would be 8.8–12.0 μg . The median daily intake of inorganic As from food and drinking water for average European consumers is estimated to be 21.0 μg for a 70 kg person (4). Yost et al. estimated daily dietary exposure to inorganic arsenic up to 14.0 and 12.7 μg in the United States and Canada, respectively (37). The above calculations suggest that wheat is the main contributor to the human intake of inorganic arsenic when the diet is not rice-based and drinking water is not contaminated with arsenic.

Supporting Information Available: Additional tables. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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