

Article

Arsenic Uptake in Durum Wheat (*Triticum durum* Desf.) as Influenced by Soil Tillage Practices and Fertilization Sources in Mediterranean Environment

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Abstract: Nowadays, arsenic (As) accumulation in agricultural soils and its transfer in crop yields is representing a growing concern that threatens food safety and security in the Mediterranean environment. Soil tillage and fertilization may increase the accumulation of As in plant tissues; therefore, there is a need to develop sustainable agronomical practices capable of supporting crop yield while mitigating As accumulation. The current study was carried out through a 7-year experiment with the aim of evaluating the As uptake by different parts of the durum wheat plant. The experimental treatments include the following: (i) three soil tillage practices (plowing, subsoiling, and spading) and (ii) two fertilization methods (mineral and organic). A factorial randomized complete block design with three replications was adopted. The experimental period refers to the 2018/2019, 2019/2020, and 2020/2021 growing seasons. The results suggest that the maximum level of As was found in plant roots and the minimum in wheat kernels. The chemical fertilization as 2020 × Mineral (1.522 mg As kg⁻¹ d.m.) and 2020 × Plowing (1.855 mg As kg⁻¹ d.m.) had the maximum As content in the roots. Conversely, the content of As was at a minimum in the wheat kernels for organic fertilization as 2021 × Organic (0.012 mg As kg⁻¹ d.m.) and subsoiling tillage as 2021 × Subsoiling (0.008 mg As kg⁻¹ d.m.). Moreover, the application of an organic fertilization source as a tool for enhancing the soil organic matter content also significantly decreased the As content. The results suggest that reduced tillage practices and the adoption of organic amendment could be classified as sustainable agronomic practices in agri-food systems, which are able to improve plant quality and assure a safe consumption of wheat kernels.

Keywords: soil tillage; soil fertilization; agri-food systems; heavy metal; As uptake



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1. Introduction

Cereal crops are an integral part of the human diet and livestock feed, necessary for fulfilling dietary needs. Some of the important cereal crops belonging to the *Graminaceae* family include wheat (*Triticum* spp.), rye (*Secale cereale*), barley (*Hordeum vulgare* L.), rice (*Oryza sativa* L.), millet (*Pennisetum glaucum* (L.) R.Br.), corn (*Zea mays* L.), and sorghum

(*Sorghum Moench*) [1]. Rice, maize, and wheat are staple food crops and are widely consumed worldwide; thus, food security is linked to the quality and production levels of these cereal crops [2,3]. The accumulation of arsenic (As) in cereal crops is a serious threat to human safety [4]. Therefore, organizations like the European Food Safety Authority (EFSA), the World Health Organization (WHO) [5], and the U.S. Food and Drug Administration (FDA) [6] have established specific regulations to control the contamination of food crops by establishing safety levels. According to WHO (2011), the weekly tolerable intake of inorganic As is $5 \mu\text{g kg}^{-1}$ body weight. For this reason, there is an immediate need to develop a sustainable cultivation system for cereal crops, including wheat.

The wheat varieties of current agro-economic interest are durum wheat (*T. turgidum durum* L.), mainly used in the pasta industry, and winter wheat (*T. aestivum* L.), used in bread and pastry-making. Under Mediterranean cropping systems, common agronomical practices are based on the adoption of intensive soil tillage for seedbed preparation and the application of high amounts of mineral fertilizers to support intensive cropping system yields. In geogenic areas with an arsenic presence, the excessive use of these agronomical practices, typically adopted by Mediterranean farmers, results in the accumulation of As in the soil. Indeed, As is naturally present in soil and underground water at variable concentrations, due to the presence of geothermal processes and *rock-aquifer* interactions. Some studies have highlighted that the accumulation of As is linked to P-based fertilizers; therefore, the application of these fertilizers increases its availability in the soil and in the plant [7,8]. Studies show that both organic and inorganic As groups have been used as pesticides on cereal crops [9]. Moreover, the uptake of As by wheat plants may result in the presence of As in wheat grains. The production and consumption of contaminated foods obtained by wheat is a threat to food security and human health, as around 85% of world population consumes wheat-derived foods for basic calories, making it an important source of energy. The presence of As in agroecosystems is challenging for wheat production and requires the adoption of adequate agronomical practices to mitigate its risks, as it is highly carcinogenic and can cause cardiovascular diseases, diabetes, and anemia [10]. In fact, the study by [11] showed that the consumption of food crops with traces of As is the second potential source of human exposure to As. Therefore, the identification of the most sustainable production practice to obtain high-quality wheat with As content below the accepted threshold for human consumption represents an important challenge in Mediterranean environments [12]. Reduced tillage practices can effectively produce high-quality crops without compromising soil health and quality. Also, the use of organic fertilizer can be a breakthrough to overcome the daunting threat of As contamination in soil and in the wheat crops. As the toxicokinetics of As in plants from the soil is dependent on soil organic matter content, soil pH, soil texture, and redox reactions. The continuous use of organic fertilizer can reduce the As concentration and consequently the plant uptake of As [13]. Therefore, the present study was designed with the objective of evaluating and identifying the most suitable agronomic practice for alleviating the As uptake level for durum wheat in the Mediterranean area. The adaptation of conservation tillage practices and organic fertilization methods in place of traditional agronomic practices could be a way forward to overcome the present and future challenges related to heavy metal uptake by cereal crops.

2. Materials and Methods

2.1. Study Site and Soil Characteristics

This study was carried out at the experimental farm “Nello Lupori” at the University of Tuscia ($45^{\circ}25' \text{ N}$ and $12^{\circ}6' \text{ E}$, 310 m a.s.l.), for three consecutive growing seasons (2018/2019, 2019/2020, and 2020/2021). The experiment is the continuation of the field study started in

2013. The experimental area is representative of wheat cultivation in the Mediterranean climate with an average air temperature of 14.5 °C and an average total annual rainfall of 752 mm. The meteorological conditions observed during the experimental periods are reported in Figure 1. The soil of the experimental site is classified as *Typic Xerofluvent*, and the surface horizon of 0–30 cm soil depth contained 760 g kg⁻¹ sand, 130 g kg⁻¹ silt, and 110 g kg⁻¹ clay (loamy sand), with a pH of 6.9. The soil had 0.97 % and 0.12 % of total organic C and N, respectively.

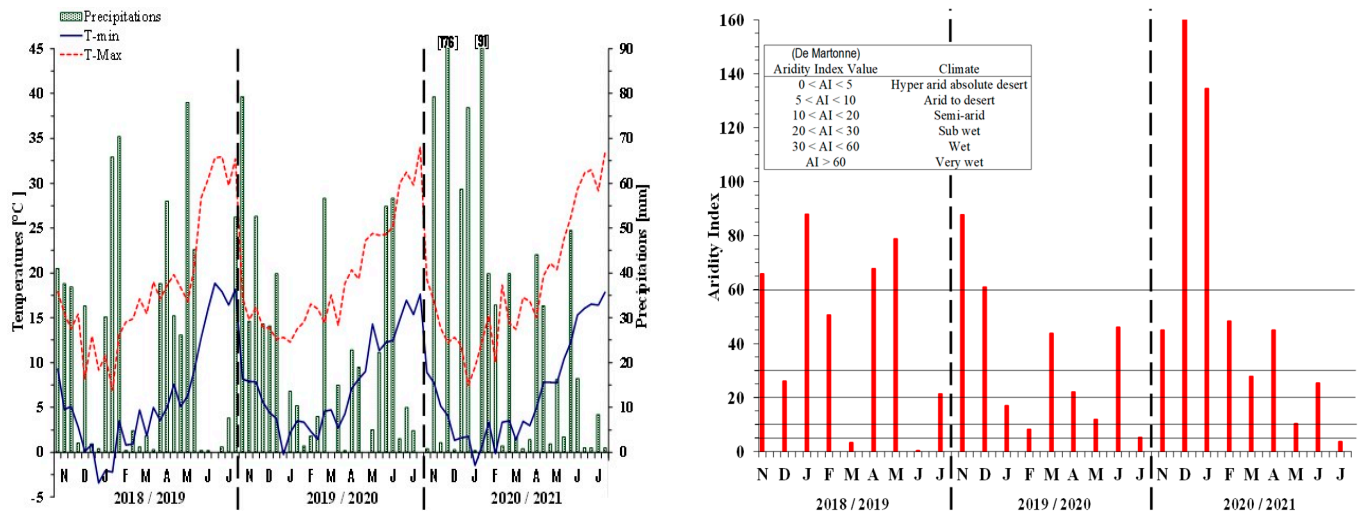


Figure 1. Decadal minimum [—] and maximum [- -] temperatures (°C), rainfall [■] (mm) at the experimental site, and aridity index (red horizontal columns) throughout the periods of study from 2018 to 2021.

2.2. Experimental Site and Design

The experimental site was established in 2013 to compare soil tillage practices and fertilizer sources in a cropping system with a 2-year crop rotation of durum wheat (*Triticum durum* Desf.)–potato (*Solanum tuberosum* L.). The adopted treatments were as follows: (a) three tillage systems [conventional tillage based on ploughing (Pl), reduced tillage based on subsoiling (Sub), and reduced tillage based on spading (Sp)]; (b) two fertilizer sources [mineral fertilization (Min) as performed in conventional farming and organic fertilization (Org) by means of municipal organic waste]. The treatments were replicated three times according to a randomized complete block design. Both crops in rotation were simultaneously cultivated each year in experimental plots 60 m² (6 × 10 m).

2.3. Field Setup and Crop Management

The durum wheat seedbed was prepared in September according to the soil tillage treatments. All tillage was carried out up to 30 cm of soil layer and then followed by a disk harrowing of up to 10 cm of soil depth. The mineral fertilizer sources were performed according to the local practices. A total of 80 kg of P₂O₅ ha⁻¹ as a triple superphosphate was applied before the last disk harrowing for seedbed preparation. A total of 100 kg of N ha⁻¹ was divided into two rates: the first N application (50 kg of N ha⁻¹ as a calcium nitrate) was carried out at the beginning of the tillering stage in February; the second N application (50 kg of N ha⁻¹ as urea) was applied at the beginning of the stem elongation in March. Chemical potassium fertilization was not applied. Regarding organic fertilizers, municipal organic waste was applied at the rate of 10,000 kg ha⁻¹ fresh weight in order to apply the same amount of nitrogen applied in the mineral fertilization treatments. The characteristics of the MOW fertilizer were moisture 50%, pH 7.3, organic carbon 25.7% of dry matter (d.m.), Organic nitrogen 2.4% of d.m., C/N ratio 10.7, and salinity 3.8 dS m⁻¹, respectively;

the complete MOW analysis showed no As content. The MOW fertilizer was applied before the last disk harrowing for the seedbed preparation. Durum wheat, cv. 'Antalis', was sown in the same day in all treatments in November by means of experimental planters (Wintersteiger, Ried inn Innkreis, Austria) at the seed density of 450 seeds m^{-2} with a row distance of 12.5 cm and about 3 cm of depth. The weeds were managed using herbicides (Mesosulfuron-Metile 3% + Iodosulfuron-Metil-Sodium 3% + Mefenpir-Dietile 9%), which were applied at the end of the wheat tillering in all treatments, as typically carried out by the local farmers. In all growing seasons, durum wheat was harvested upon physiological maturity of the kernels at the end of June.

2.4. Sample Preparation and Arsenic Analysis

At harvesting time, the wheat above and the biomass below ground were manually sampled from three 1 m long adjacent rows in the middle of each plot. Then, in the laboratory, each part of the plants was separated, and the yield components were determined. The samples were oven-dried at 65 °C until constant to determine the dry weight.

At harvesting, soil samples were randomly taken at a depth of 0–30 cm in five points and mixed to obtain a representative sample in each plot. The soil samples were air-dried, sieved with a 2 mm mesh sieve, and then analyzed.

The wheat and soil sample analyses were carried out in the Laboratory of Commodities and Territorial Analysis of the University of Cassino and Southern Lazio. The As determination in wheat was performed separately in the kernels, stems, leaves, and roots collected. The soil analyses were carried out using 0.2–0.3 g of samples mineralized through a wet digestion process in the presence of a mixture of 3 mL of nitric acid (HNO_3 65% RS) and 0.5 mL of a solution of hydrogen peroxide (H_2O_2 40% m/V in purely stabilized water) supplied by Carlo Erba Reagents. After the acid digestion, ultrapure distilled water HIGH PURITY 18 $\text{M}\Omega$ cm^{-1} 25 °C was used for the recovery of the samples, and the mixture was brought to a final volume of 10 mL. The presence of As was determined by an Atomic Absorption Spectrophotometer AA-600 with the hydride generation system FIAS-100 (Perkin Elmer, Springfield, IL, USA), with the instrument detection limit on the GFAA of 1 ppb. The calibration was made using appropriate dilutions of the stock solution of As at 1.000 ± 0.002 g L^{-1} in 2% HNO_3 (CPAchem, Bogomilovo, Bulgaria), and to ensure the reproducibility and the accuracy of the method, the same analyses were conducted on a standard reference material, NIST 1570a (trace element in spinach leaves, Sigma Aldrich, Taufkirchen, Germany) with a mean recovery of about $95 \pm 1\%$. In the graphite furnace, 20 μL of sample was introduced, and subsequently the sample was atomized according to a specific temperature program. The As concentration was expressed in milligrams per kilo of dry weight (mg kg^{-1} dw).

2.5. Bioaccumulation Factors (BAFs)

The As Bioaccumulation Factors (BAFs) for each sample were calculated to evaluate the ability of the plant to accumulate As. As indicated by Dessalew et al. (2018), the BAFs were calculated using the following formulas:

$$\text{BAFr}_s = \text{Croot}/\text{Csoil};$$

$$\text{BAF}_{ss} = \text{Cstem}/\text{Csoil};$$

$$\text{BAF}_l = \text{Cleaf}/\text{Csoil};$$

$$\text{BAF}_{gs} = \text{Cgrain}/\text{Csoil};$$

where BAFrs, BAFss, BAFls, and BAFgs, were the Bioaccumulation Factors in roots, stems, leaves, and grains, respectively; and Croot, Cstem, Cleaf, and Cgrain were the As concentrations in the roots, stems, leaves, and grains, respectively.

2.6. Statistical Analysis

The statistical analyses were performed using the JMP statistical software package version 4.0 (Littel et al., 1996). The analysis of variance (ANOVA) was carried out using the ANOVA model with the treatments as a fixed factor, the three blocks were included as a random factor, and the growing season was considered as a random effect to account for the repeated measure across time. Fisher's protected least significant difference (LSD) at the 0.05 probability level ($p < 0.05$) was used for comparing the main effects. Linear regressions were performed for selected variables.

3. Results

3.1. Arsenic Translocation in Different Parts of Wheat Plant

According to Figure 2, the As content in the different plant sections were significantly different from each other. The roots had the maximum content of As, whereas the kernel had the minimum. The results shown in Table 1 suggest that a significant difference was observed between the different growing seasons \times tillage for As in soil. The maximum level of soil As content was observed in 2020/2021_Plo followed by 2020/2021_Spa and 2020/2021_Sub (0.552, 0.391, and 0.213 mg As kg⁻¹, respectively), whereas the minimum level of As was observed in 2018/2019_Plo (0.096 mg As kg⁻¹). There was a significant difference between 2020/2021_Plo and all other treatments. However, 2020/2021_Spa and 2020/2021 \times Sub had no significant difference between each other, but they were different from other treatments. Results also show that the interaction between growing seasons and fertilization was also significantly different from each other. The maximum soil As content was observed in 2020/2021_Min followed by 2019/2020_Min (0.491 and 0.295 mg As kg⁻¹, respectively). These treatments were significantly different from each other and organic fertilization. The minimum level of soil As for fertilization was observed in 2018/2019_Org (0.113 mg As kg⁻¹), and it showed no major difference from the organic treatments of the 2019/2020 and 2020/2021 growing seasons. Moreover, there was an important interaction between soil tillage \times fertilization source. The results showed a maximum level of soil As in Spa_Min and a minimum level in Spa_Org (0.491 and 0.138 mg As kg⁻¹, respectively). However, there was no significant difference observed among other interactions ($p > 0.05$).

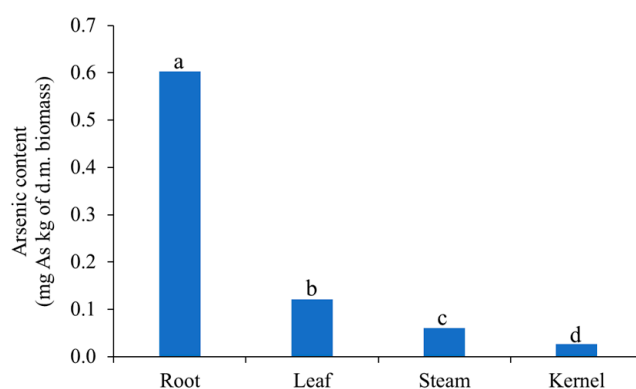


Figure 2. Arsenic content in each part of the plant. Values with different letters are statistically different according to LSD (0.05). Values of bars with different letters are statistically different according to LSD (0.05).

Table 1. Arsenic content in the soil, interactions growing season \times soil tillage, growing season \times Fertilization source, soil tillage \times fertilization source. Values with different letters in each group are statistically different according to LSD (0.05).

Treatments	Treatments	Soil As (mg As kg ⁻¹ of Soil)	
Year	Soil tillage		
2018/2019	Plowing	0.096	d
2018/2019	Subsoiling	0.106	d
2018/2019	Spading	0.202	c
2019/2020	Plowing	0.166	c,d
2019/2020	Subsoiling	0.207	c
2019/2020	Spading	0.350	b
2020/2021	Plowing	0.552	a
2020/2021	Subsoiling	0.213	c
2020/2021	Spading	0.391	b
Year	Fertilization		
2018/2019	Mineral	0.157	c,d
2018/2019	Organic	0.113	d
2019/2020	Mineral	0.295	b
2019/2020	Organic	0.187	c
2020/2021	Mineral	0.595	a
2020/2021	Organic	0.176	c,d
Soil tillage	Fertilization		
Plowing	Mineral	0.395	b
Plowing	Organic	0.147	c
Subsoiling	Mineral	0.160	c
Subsoiling	Organic	0.191	c
Spading	Mineral	0.491	a
Spading	Organic	0.138	c

The results showed that the concentration of As in the kernel decreased in the order 2020/2021_Spa > 2018/2019_Sub (0.076 and 0.037 mg As kg⁻¹ d.m., respectively). Whereas the minimum value was observed in 2020/2021_Sub (0.008 mg As kg⁻¹ d.m.) and 2019/2020_Spa (0.008 mg As kg⁻¹ of d.m.) without any significant difference between them, they differed from other interactions.

The results reported in Table 2 show that the growing season \times fertilization source interaction was significantly higher in 2020/2021_Min followed by 2018/2019 \times Min (0.061 and 0.030 mg As kg⁻¹ d.m., respectively). The minimum concentration of As in the kernel was observed in 2020/2021_Org (0.012 mg As kg⁻¹ d.m.). According to the results, 2020/2021_Min showed a major difference from other treatments, whereas there was no significant difference observed among the remaining treatments. The interaction between soil tillage \times fertilization source is also shown in Table 2. The maximum As concentration was shown by Spa_Min (0.63 mg As kg⁻¹ d.m.), and it was also significantly different from other interactions, whereas minimum As concentration in kernel was observed in Spa_Org (0.013 mg As kg⁻¹ d.m.).

Table 2. Arsenic content in the wheat plant kernel, interactions growing season × soil tillage, growing season × fertilization source, soil tillage × fertilization source. Values with different letters in each group are statistically different according to LSD (0.05).

Treatments	Treatments	Kernel As (mg As kg ⁻¹ d.m.)	
Year	Soil tillage		
2018/2019	Plowing	0.014	d,e
2018/2019	Subsoiling	0.037	b
2018/2019	Spading	0.030	b,c
2019/2020	Plowing	0.023	c,d
2019/2020	Subsoiling	0.020	c-e
2019/2020	Spading	0.008	e
2020/2021	Plowing	0.026	b-d
2020/2021	Subsoiling	0.008	e
2020/2021	Spading	0.076	a
	<i>p</i> value	<0.0001	
Year	Fertilization		
2018/2019	Mineral	0.030	b
2018/2019	Organic	0.024	b,c
2019/2020	Mineral	0.015	c,d
2019/2020	Organic	0.019	b-d
2020/2021	Mineral	0.061	a
2020/2021	Organic	0.012	d
	<i>p</i> value	<0.0001	
Soil tillage	Fertilization		
Plowing	Mineral	0.020	b
Plowing	Organic	0.023	b
Subsoiling	Mineral	0.024	b
Subsoiling	Organic	0.020	b
Spading	Mineral	0.063	a
Spading	Organic	0.013	b
	<i>p</i> value	<0.0001	

The concentration of As in wheat stems is shown in Table 3. The interaction between 2019/2020_Spa (0.148 mg As kg⁻¹ d.m.) had the maximum As content in the stem followed by 2019/2020_Plo (0.099 mg As kg⁻¹ d.m.) and 2019/2020_Sub (0.086 mg As kg⁻¹ d.m.), respectively. The 2019/2020_Spa showed a significant difference from other tillage practices within the same growing season. The minimum As concentration in the stems was found in 2020/2021_Plo (0.051 mg As kg⁻¹ d.m.). Furthermore, the growing season × fertilization source interaction had the maximum value for 2019/2020_Org followed by 2019/2020_Min and 2020/2021_Org (0.139, 0.083, 0.049 mg As kg⁻¹ d.m., respectively). The 2018/2019_Min had the minimum concentration (0.051 mg As kg⁻¹ d.m.) of As in the stem. The lettering suggests that 2019/2020_Org and 2019/2020_Min interactions were majorly different from others. The most significant interaction with the highest value was observed in Spa_Org (0.139 mg As kg⁻¹ d.m.). The interaction between Sub_Min had the minimum As concentration level in the plant stem (0.047 mg As kg⁻¹ d.m.).

Table 3. Arsenic content in the plant stem, interactions growing season × soil tillage, growing season × fertilization source, soil tillage × fertilization source. Values with different letters in each group are statistically different according to LSD (0.05).

Treatments	Treatments	Stem As (mg As kg ⁻¹ d.m.)	
Year	Soil tillage		
2018/2019	Plowing	0.023	d
2018/2019	Subsoiling	0.031	d
2018/2019	Spading	0.022	d
2019/2020	Plowing	0.099	b
2019/2020	Subsoiling	0.086	b
2019/2020	Spading	0.148	a
2020/2021	Plowing	0.051	c
2020/2021	Subsoiling	0.035	c,d
2020/2021	Spading	0.052	c
	<i>p</i> value	<0.0001	
Year	Fertilization		
2018/2019	Mineral	0.021	e
2018/2019	Organic	0.029	d,e
2019/2020	Mineral	0.083	b
2019/2020	Organic	0.139	a
2020/2021	Mineral	0.043	c,d
2020/2021	Organic	0.049	c
	<i>p</i> value	<0.0001	
Year	Fertilization		
Plowing	Mineral	0.048	c
Plowing	Organic	0.068	b
Subsoiling	Mineral	0.047	c
Subsoiling	Organic	0.055	b,c
Spading	Mineral	0.053	b,c
Spading	Organic	0.095	a
	<i>p</i> value	<0.0172	

The As content in the leaf suggests that only plowing and spading for the 2019/2020 growing season showed a significant difference from other treatments but not between each other. However, a maximum concentration of As in the wheat leaf was shown by 2019/2020_Plo (0.295 mg As kg⁻¹ d.m.) and minimum by 2018/2019_Sub (0.040 mg As kg⁻¹ d.m.). The concentration of As in the leaf for growing season × fertilization showed no significant differences except in 2019/2020 (Table 4). The highest value was observed in 2019/2020_Org followed by 2019/2020_Mineral (0.217 and 0.203 mg As kg⁻¹ d.m., respectively). The third interaction between tillage × fertilization showed a maximum As content in Plo_Org followed by Spa_Min and Plo_Min (0.223, 0.189 and 0.138 mg As kg⁻¹ d.m., respectively). The subsoiling and spading tillage interaction with both fertilizations had no significant differences (Table 4).

Table 4. Arsenic content in the plant leaf, interactions growing season \times soil tillage, growing season \times fertilization source, soil tillage \times fertilization source. Values with different letters in each group are statistically different according to LSD (0.05).

Treatments	Treatments	Leaf As (mg As kg ⁻¹ d.m.)	
Year	Soil tillage		
2018/2019	Plowing	0.123	b
2018/2019	Subsoiling	0.040	b
2018/2019	Spading	0.044	b
2019/2020	Plowing	0.295	a
2019/2020	Subsoiling	0.064	b
2019/2020	Spading	0.270	a
2020/2021	Plowing	0.123	b
2020/2021	Subsoiling	0.064	b
2020/2021	Spading	0.061	b
	<i>p</i> value	<0.0166	
Year	Fertilization		
2018/2019	Mineral	0.075	b
2018/2019	Organic	0.063	b
2019/2020	Mineral	0.203	a
2019/2020	Organic	0.217	a
2020/2021	Mineral	0.109	b
2020/2021	Organic	0.056	b
	<i>p</i> value	<0.0489	
Soil tillage	Fertilization		
Plowing	Mineral	0.138	b
Plowing	Organic	0.223	a
Subsoiling	Mineral	0.060	c
Subsoiling	Organic	0.052	c
Spading	Mineral	0.189	a
Spading	Organic	0.061	c
	<i>p</i> value	<0.0011	

The As content in wheat roots is shown in Table 5. According to the results for growing season \times soil tillage, the 2019/2020_Plo followed by 2019/2020_Spa and 2019/2020_Sub (1.855, 1.446, and 0.766 mg As kg⁻¹ d.m.) showed significantly higher different values than all other interactions. The As content for growing season \times fertilization showed significant results for 2019/2020_Min and 2019/2020 \times Org (1.522 and 1.190 mg As kg⁻¹ d.m., respectively). The soil tillage \times fertilization interaction was significantly higher and different in Plo_Org and Spa_Min interactions. Moreover, the minimum As values in the root were observed by Spa_Org (0.171 mg As kg⁻¹ d.m.), even if there was no significant difference from other treatments expected for the highest value interactions.

3.2. Accumulation of As in Wheat Plant and Soil

The grain yield/grain As (Table 6) showed no significant differences among all of the treatments for growing season \times soil tillage, except for 2019/2020 \times Spa (56896) and 2020/2021_Sub (39034). The spading tillage in 2019/2020 had the highest value of As in the grains, and the minimum value was shown by plowing tillage in 2020/2021. Also, the As content ratio in grains for growing season \times fertilization was calculated with less significant differences among treatments. However, 2019/2020_Min (39579) had the highest grain yield/grain As ratio compared to other treatments, and 2018/2019_Org (9471) had the minimum As ratio in the grains. The results also showed no significant differences in As ratio for soil tillage \times fertilization interactions, except for Spa_Org (36865).

Table 5. Arsenic content in the root of the wheat plant, interactions growing season × soil tillage, growing season × fertilization source, soil tillage × fertilization source. Values with different letters in each group are statistically different according to LSD (0.05).

Treatments	Treatments	Root As (mg As kg ⁻¹ d.m.)	
Year	Soil tillage		
2018/2019	Plowing	0.452	d
2018/2019	Subsoiling	0.354	d
2018/2019	Spading	0.216	d,e
2019/2020	Plowing	1.855	a
2019/2020	Subsoiling	0.766	c
2019/2020	Spading	1.446	b
2020/2021	Plowing	0.217	d,e
2020/2021	Subsoiling	0.063	e
2020/2021	Spading	0.058	e
	<i>p</i> value	<0.0001	
Year	Fertilization		
2018/2019	Mineral	0.337	c
2018/2019	Organic	0.344	c
2019/2020	Mineral	1.522	a
2019/2020	Organic	1.190	b
2020/2021	Mineral	0.061	d
2020/2021	Organic	0.164	c,d
	<i>p</i> value	<0.0294	
Soil tillage	Fertilization		
Plowing	Mineral	0.412	c,d
Plowing	Organic	1.271	a
Subsoiling	Mineral	0.532	c,d
Subsoiling	Organic	0.257	d,e
Spading	Mineral	0.975	b
Spading	Organic	0.171	e
	<i>p</i> value	<0.0001	

The aboveground biomass for the growing season and fertilization was significant and different, whereas the soil tillage produced no major difference among treatments (Table 7). The maximum aboveground biomass was observed in 2019/2020 (906.6 g m⁻²), Min (821.4 g m⁻²), and Sub (791.1 g m⁻²). The concentration of As uptake by the grain was not significantly different for different growing seasons. The spading tillage (0.0095 g m⁻²) showed significantly high levels and was different compared to other plowing and subsoiling. Moreover, Min (821.4 g m⁻²) had the highest and most significantly different amount of As uptake by the grain as compared to Org (0.0047 g m⁻²). The results for the soil TOC for growing season and fertilization treatment were significant and different. However, for the tillage treatments, only spading had a significantly different value compared to plowing and subsoiling. The maximum soil TOC was produced by 2019/2020 (1.185%) followed by 2018/2019 and 2020/2021, respectively. The percentage of soil TOC was high in Org (0.988%) as compared to Min. However, Sub (0.988%) and Spa (1.008%) had no significant difference between each other for the soil TOC, and plowing (0.906%) showed the minimum percentage of the soil TOC. Moreover, the results shown for the soil TON were only majorly different for 2018/2019 (0.103%).

Table 6. Arsenic content in the grain yield/grain As of the wheat plant, interactions growing season × soil tillage, growing season × fertilization source, soil tillage × fertilization source. Values with different letters in each group are statistically different according to LSD (0.05).

Treatments	Treatments	Grain Yield/Grain As Ratio (g m ⁻² /mg kg ⁻¹)	
Year	Soil tillage		
2018/2019	Plowing	18,809	d
2018/2019	Subsoiling	9685	d
2018/2019	Spading	9238	d
2019/2020	Plowing	19,323	c,d
2019/2020	Subsoiling	20,299	c,d
2019/2020	Spading	56,896	a
2020/2021	Plowing	8520	d
2020/2021	Subsoiling	39,034	b
2020/2021	Spading	31,105	b,c
	<i>p</i> value	<0.0001	
Year	Fertilization		
2018/2019	Mineral	15,684	c,d
2018/2019	Organic	9471	d
2019/2020	Mineral	39,579	a
2019/2020	Organic	24,766	b,c
2020/2021	Mineral	22,616	b,c
2020/2021	Organic	29,824	a,b
	<i>p</i> value	<0.0125	
Soil tillage	Fertilization		
Plowing	Mineral	21,564	b,c
Plowing	Organic	9538	d
Subsoiling	Mineral	28,353	b
Subsoiling	Organic	17,659	c,d
Spading	Mineral	27,962	b
Spading	Organic	36,865	a
	<i>p</i> value	<0.0034	

The BAFrs for growing season × soil tillage interaction was very high in 2019/2020_Plo (12.996) (Table 8). Whereas the growing season × fertilization interaction for BAFrs had no great difference among all of the treatments. The significantly different results for soil tillage × fertilization interactions were observed in Plo_Org (9.867) and Sub_Min (6.611). BAFss had a higher value in 2019/2020_Sub (0.539) compared to other interactions. The results for growing season × fertilizer suggest that 2018/2019 × organic (0.271) had the maximum, and 2020/2021_Min (0.089) showed a minimum amount of BAFss. The interaction between soil tillage × fertilization was significantly higher in Spa_Org (0.816) and lowest in Spa_Min (0.102). For BAFIs, the plowing tillage in 2019/2020 (2.188) and 2018/2019 (2.122) had higher values as compared to other interactions, but they showed no significant difference between each other. The interaction between growing season × fertilizer showed the maximum value for BAFIs in 2019/2020_Org (1.618). The Plo_Org (1.848) interaction also showed the maximum value for BAFIs and was significantly different from other interactions, except for Plo_Min (1.185). The BAFgs had the highest value in 2018/2019_Spa (0.191) as compared to other interactions, and it also showed a major difference from other interactions. Whereas Min (0.320) and Org (0.241) fertilizer in the growing season 2018/2019 had no differences between each other but showed significantly high and diverse results for BAFgs. The soil tillage and fertilization interaction had no major effect on the BAFgs. However, the maximum value was observed in Sub_Min

(0.260). The comparative results for soil Carbon % and As content in kernel as affected by fertilization show that with an increase in soil C %, the As content in the wheat kernels greatly decreased in organic fertilization, whereas the As content in kernel did not show any significant change with the increase in soil Carbon % (Figure 3).

Table 7. Aboveground biomass of wheat crop, uptake of As by the wheat grain, soil total organic carbon, soil total organic nitrogen, as affected by the growing season, soil tillage, and fertilization source. Values with different letters in each group and in each parameter are statistically different according to LSD (0.05).

Treatments	Aboveground Biomass (g DM m ⁻²)	Up Taken As by Grain (mg m ⁻²)	Soil TOC (%)	Soil TON (%)
Year				
2018/2019	596.3 c	0.0073 a,b	0.963 b	0.103 b
2019/2020	906.6 a	0.0066 b	1.185 a	0.139 a
2020/2021	727.1 b	0.0083 a	0.754 c	0.149 a
Soil tillage				
Plowing	680.8 b	0.0055 b	0.906 b	0.121 a
Subsoiling	791.1 a	0.0071 b	0.988 a	0.129 a
Spading	758.0 a,b	0.0095 a	1.008 a	0.142 a
Fertilization				
Mineral	821.4 a	0.0100 a	0.947 b	0.133 a
Organic	665.3 b	0.0047 b	0.988 a	0.128 a

TOC = total organic carbon; TON= total organic nitrogen.

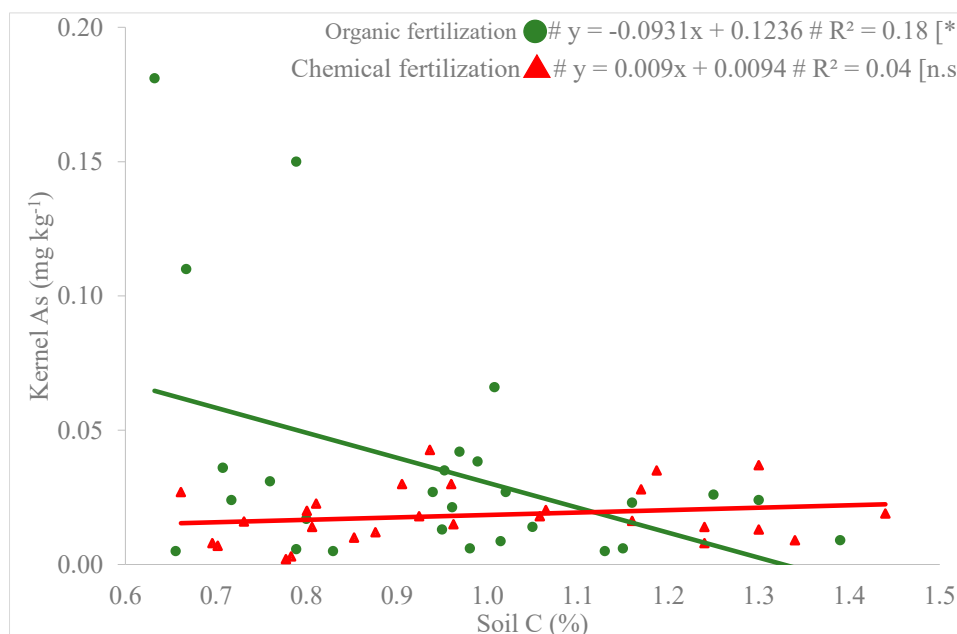


Figure 3. Soil carbon content plotted against arsenic content in the kernel as affected by fertilization. In the square brackets, * and n.s. represent significance for $p < 0.05$ and no significance, respectively, according to an ANOVA test.

Table 8. Arsenic Bioaccumulation Factors (BAFs), interactions growing season \times soil tillage, growing season \times fertilization source, soil tillage \times fertilization source. Values with different letters in each group and in each parameter are statistically different according to LSD (0.05).

Treatments	Treatments	BAFr _s	BAF _{ss}	BAF _l	BAF _{gs}
Year	Soil tillage				
2019	Plowing	6.724 b	0.340 c	2.122 a	0.225 b
2019	Subsoiling	5.640 c,d	0.317 e	0.524 b	0.425 b,c
2019	Spading	1.806 b,c	0.144 c,d	0.312 b	0.191 a
2020	Plowing	12.996 a	0.658 b	2.188 a	0.156 b,d
2020	Subsoiling	6.650 b,d	0.539 a	0.459 b	0.152 d
2020	Spading	3.099 b	0.999 b	0.720 b	0.051 b,d
2021	Plowing	0.906 d	0.164 d,e	0.240 b	0.067 c,d
2021	Subsoiling	0.373 d	0.181 c,e	0.322 b	0.048 b,d
2021	Spading	0.223 d	0.235 c,e	0.237 b	0.131 d
Year	Fertilization				
2019	Mineral	5.524 a,b	0.262 b	1.267 a,b	0.320 a
2019	Organic	3.922 b,c	0.271 a	0.706 b,c	0.241 a
2020	Mineral	6.736 a,b	0.445 c	0.626 b,c	0.113 b
2020	Organic	8.427 a,b	1.019 c	1.618 a	0.127 b
2021	Mineral	0.121 d	0.089 d	0.193 c	0.098 b
2021	Organic	0.880 c,d	0.297 c	0.339 c	0.066 b
Soil tillage	Fertilization				
Plowing	Mineral	3.882 c	0.277 c	1.185 a,b	0.124 b
Plowing	Organic	9.867 a	0.498 b	1.848 a	0.174 a,b
Subsoiling	Mineral	6.611 b	0.417 b,c	0.537 b,c	0.260 a
Subsoiling	Organic	1.831 c	0.274 c	0.333 c	0.157 a,b
Spading	Mineral	1.888 c	0.102 d	0.363 c	0.147 a,b
Spading	Organic	1.531 c	0.816 a	0.482 c	0.102 b

Bioaccumulation Factors (BAFs): rs = root; ss = stem; ls = leaf; gs = grain.

4. Discussion

The results of the present study showed how the differences in As bioaccumulation varied significantly among the different wheat plant parts. The results indicated that the concentrations of As decreased in the order of root > leaf > stem > grain, indicating that the roots act as a barrier for metal translocation and protect the edible parts from As contamination, confirming the findings of other similar studies [14,15]. The concentrations of As in grains and stems were significantly lower than those in other tissues ($p \leq 0.05$), but no major differences were found between As concentrations in grains and stems ($p > 0.05$). Many studies investigated the regularity of migration and accumulation of heavy metals between soil and crops. A previous study identified an inter- and intraspecific variation in Cd accumulation in cereal crops and concluded that Cd was more readily accumulated to higher levels than As and Pb in wheat [16]. The results show that the average As content in soil ranges from 0.07 to 1.1 mg kg⁻¹. The accumulation of As in wheat could be favored by factors such as the cultivated species and cultivation method. In reference to agronomic practices, soil compaction and irrigation were evaluated from previous studies. In compacted soil, the porosity and the air space between the particles is reduced, with an increase in the diffusion coefficient of the ions and a greater root–soil contact, which facilitates the content of nutrients. Many authors agree that the transfer of metals from the soil to the grains involves several steps, including uptake by the roots, vacuolar sequestration in the roots, translocation from the roots to the shoots, and distribution to the grains [16,17]. All of these steps are based on various transport mechanisms and by metal

chelating agents by xylem and phloem flows [18]. The results suggest that plowing tillage and chemical fertilization in 2020/2021 had the maximum As content in the soil. Previous studies show that the high level of soil disturbance could have led to an increased exposure of the already existing As in soil minerals or organic matter and also increased its mobility and availability in the soil [19]. Tilling methods significantly alter physical and chemical soil properties and may result in a decrease in the quantity of organic matter, pH, alteration of the composition of the organism communities present in the soil, and a reduction of biodiversity of soil species [20]. Intensive tillage increases the soil aeration; thus, the oxidation of As-bearing minerals increases [21]. The maximum level of As content in the soil was found in the results by the interaction of spading tillage \times mineral fertilization. Even if the soil spading tillage better manages the crop residues, mixing them along the tilled layer does not create the typical compact layer at the bottom of the working depth. In fact, it increases bulk density, decreases mean clod size, and generally causes higher soil disturbance compared to the other conventional soil tillage techniques [22]. Tillage methods can change the pH and availability of heavy metals in soils. In some cases, soil disturbance can induce an increase in the availability of these heavy metals [23]. Similar results were observed in rice and the study showed that the application of chemical fertilizers can alter soil chemistry [24]. Some studies showed that the application of phosphate fertilizers can also alter the soil pH and the redox potential and enhance the mobility and solubility of As in the soil, which makes it more bioavailable to plants and increases its concentration in the soil [25]. The mineral fertilizers also contain As as a contaminant, thus increasing the soil's As content [26]. Similarly, the combination of spading and plowing soil tillage with mineral fertilization also showed a high As content in the soil. The high As content increased the uptake by the wheat plant and resulted in an increased amount of As content in the kernel for spading tillage and mineral fertilization in the year 2020/2021. The repeated application of the same tillage and fertilization practice for 3 years aggravated the effect of soil tillage on the As availability [11], whereas the minimum level of As was shown by subsoiling tillage and organic fertilization in year 2020/2021, and the interaction of spading \times organic also had the same effect. On one side, organic fertilization determined a general increase in the soil's organic carbon, which probably affected the As content, while on the other side with a mineral fertilization combined with more soil disturbance, the As results were higher, likely because of the enhancement of the As soil mobility [27]. Therefore, the As soil and plant uptake could depend on the adopted agronomic practices related to soil fertilization and soil tillage methods. The bioavailability of As was probably changed by organic fertilizer through the alteration of the soil organic matter content. The increase in organic matter degradation can determine an increase in functional groups (carbonyl and phenolic-OH) and thus of the binding trace elements. Moreover, the addition of trace element sorbents (Fe, Mn, Al oxide) could bind with trace elements and then decrease the exchangeable fractions [28]. The reduced As uptake in the plant was due to the reduced As in the soil after organic fertilization (Wan et al., 2020). Several studies showed that the increase in soil organic matter can reduce the bioavailability of As in soils due to the adsorption or the forming of stable complexes with humic substances [28–30].

Another previous study [31] showed that subsoiling tillage helps to improve soil health by reducing runoff, increasing aeration, enhancing porosity, and decreasing bulk density. As a result, water infiltration helps in the removal of As content from the root zone [32]. Moreover, the application of organic fertilizer also improves soil microbial activity and organic matter along with maintaining a neutral pH [33]. All of these factors reduce the availability of As. The nutrient competition between phosphate present in organic fertilizer and As in soil also reduces the plant uptake of As, hence decreasing the amount present in the wheat kernel [34].

The accumulation of As in wheat stems and leaves showed the opposite trend as compared to the kernel. The maximum values were observed in the year 2019/2020 for spading tillage and organic fertilization. Moreover, their combination also had an increasing effect on the As content in the stems. Previous studies suggest that the availability of high organic matter in the soil can also improve the solubility of As by making soluble organic As complexes. This phenomenon can facilitate the uptake of nutrients by the vascular system and then accumulate them in stems and leaves [35]. Ref. [36] studied that the improved root system and increased biomass can also increase the As translocation in stems through better transpiration and water uptake. Furthermore, the As in the roots was significantly higher in the year 2020 with plowing tillage and chemical fertilization. The roots are the key plant parts responsible for the uptake of nutrients from the soil; thus, the presence of high levels of As in the soil for plowing tillage and mineral fertilization resulted in a greater As content in the roots [37].

The interaction between spading \times organic had a higher grain yield/grain As ratio, possibly because of better decomposition and availability of organic matter due to favorable environmental conditions. The results shown in Table 7 also suggest that the soil TOC and TON were also found to be relatively high in 2019/2020 spading tillage and organic fertilization. For this reason, the ratio of grain yield/grain As was also high in the respective year. Moreover, the results of the experiment also showed the effect of organic and chemical fertilization on the accumulation of As in the soil. According to the results, the effect of organic fertilizer on As accumulation was significant as compared to mineral fertilization. The content of As decreased with the application of organic fertilizer. The reduction is due to the increased organic matter that binds As and makes it less bioactive. Similar results were observed in other studies through the application of organic fertilizers and suggested that the change in the chemical properties of soil as a result of the increased production of As reducing bacteria, along with changes in pH, affect the availability of As in soil [38,39].

5. Conclusions

The results showed that the application of sustainable agronomic practices can effectively improve the quality of wheat grains by minimizing the As accumulation in kernels. The organic fertilization and subsoiling tillage seem to be the most suitable agronomic practices to reduce As content level in the kernel. It is therefore necessary to remodulate agri-food systems according to agroecological approaches, through cultivation techniques that enhance and protect natural resources and biodiversity. Sustainable agronomic techniques (such as spading tillage and organic composted fertilizer) showed different positive effects, such as increasing soil organic matter, reduced requirement of energy, and agroecological value of crop rotation. In these environmental conditions, relative to the As soil accumulation and plant uptake, the cropping system is favored by reduced soil tillage through subsoiling and organic fertilization. Therefore, it can be concluded that the adaptation of sustainable agronomic practices is imperative to the production of wheat in the Mediterranean environment to ensure food safety and high-quality wheat production for human consumption. In both applied treatments (soil tillage and fertilization), the kernels have not exceeded the thresholds for As provided by the limits suggested by the European Food Safety Authority [40].

Further studies will be required to explore the effectiveness of these agronomical practices and how this kind of organic compost can affect other crops' As uptake, such as the most common vegetables (potato or tomato), with different tillage methods, also studying how the soil microbial pool interact with As and soil organic biomass.

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