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Comparative analysis of wheat heavy metal accumulation across multiple regions and the influence of cultivation practices

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ABSTRACT

Metal (loid) contamination in food poses a significant global health concern and represents a long-term threat to crop growth and agricultural sustainability. This study focused on the Henan Province of China (Yuzhou and Jiyuan) and the Marche region of Italy (Jesi and Camerino) to analyze the uptake and translocation characteristics of heavy metals (HMs) in wheat across different regions. Additionally, it employed chemometric and transcriptomic techniques to compare the effects of organic (Org) and conventional (Conv) cultivation. Results showed significant regional differences in soil pollution profiles: Pb and Cd dominated in Jiyuan, Cd in Yuzhou, and Al and Cu in Jesi. The bioaccumulation factors (BAF) of Cr, Ni, and As in wheat grains were significantly higher in the two locations in Henan compared to those in Marche, indicating a greater propensity for these metals to be translocated and accumulated in the grains. During the tillering stage, wheat actively absorbed and translocated Mn, Al, and Cd, whereas Pb, As and Ni accumulated predominantly during the filling stage, demonstrating stage-specific responses of wheat to HM stress. Org reduced soil HM contents by an average of 1.29 % annually compared to Conv. Wheat grown near railways (Org-rial) exhibited an average increase of 21.25 % in HM contents, indicating a stimulatory effect of railway pollution on HM uptake and accumulation. Transcriptomic analysis identified 25,102 differentially expressed genes in wheat leaves under Org. Org significantly enhanced the expression of genes involved in metabolic pathways, photosynthesis, and antioxidant mechanisms, while suppressing the expression of certain HM transport-related genes. This study underscores the potential of Org to mitigate HM accumulation in crops, contributing to enhanced food safety and sustainable agricultural practices.

1. Introduction

With the rapid development of modern society, various environmental pollution issues have become increasingly prevalent. As a significant type of environmental pollution, the harmful effects of heavy metals (HMs) pollution have gained attention in various fields such as ecology (Wang et al., 2023), plant nutrition (Xu et al., 2021), and agriculture (Yang et al., 2018). According to FAO estimates, global wheat production will reach approximately 787 million tonnes, with China and Italy contributing about 140 and 6.1 million tonnes, respectively (FAOSTAT database: https://www.fao.org/faostat/en/#data/QCL). As a key staple crop, wheat not only serves as the primary raw material for a wide range of food products such as noodles, bread, and pasta, but also acts as a cornerstone of food security, playing a critical role in agricultural production in China and Italy (Jiang et al., 2020). However, the environmental accumulation of HMs poses a dual threat: by disrupting enzymatic activity and photosynthesis, it impairs crop growth, and subsequently, through metal-specific transporters and chelation mechanisms, HMs are translocated via the xylem and phloem to edible tissues, leading to long-term health risks upon entry into the food chain (Chen et al., 2023; Di et al., 2025; Kasiviswanathan et al., 2024). In addition to industrial activities such as smelting, manufacturing, construction, and transportation, HM deposition through evaporation and precipitation, as well as discharges from domestic wastewater and industrial effluents, are also significant sources of wheat contamination (Hussain et al., 2021). These varied sources of pollution lead to region-specific and cultivation-dependent patterns of HM accumulation in wheat (Dagdag et al., 2023; Li et al., 2023). Nevertheless, systematic comparative studies on HM content in

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wheat from different countries and cultivation practices remain relatively scarce. Although existing studies have revealed the regulatory mechanisms of various trace elements, such as selenium, in mitigating HM accumulation in crops (Di et al., 2025), most focus on the mechanisms of individual elements or the effects under short-term treatment conditions. The interactions among HMs significantly influence their absorption and translocation in plants, with these effects varying in direction and magnitude depending on metal types, contents, and environmental conditions (Xu et al., 2022; Mwamba et al., 2016; Ran et al., 2016). In cucumber (Cucumis sativus), varying levels of Cu and Pb were added to the soil, which resulted in opposite regulatory effects: Cd accumulation was stimulated by low levels of Pb ($<160\ mg\cdot kg^{-1}$), whereas high levels of Pb ($160–640\ mg\cdot kg^{-1}$) suppressed Cd accumulation (An et al., 2004). However, wheat displayed differential responses to HMs stress, with Cd exhibiting the greatest toxicity and mobility. Pb mitigated Cd toxicity via competitive absorption, whereas Ni acted synergistically with Cd to intensify oxidative damage (Saleh et al., 2020). These synergistic and antagonistic interactions exhibit strong concentration and environment dependence, creating a complex regulatory landscape for HM accumulation in plants.

Beyond environmental factors, cultivation practices are fundamental to agricultural management. Liu et al. (2023) reported that different cultivation systems (Org and Conv, from 2016 to 2021) influence the bioavailability, mobility, and uptake of HMs in plants through multi-level mechanisms. These mechanisms include modifications of soil properties (such as pH and temperature), alterations in rhizosphere microbial communities, and changes in plant metabolic pathways (Uchimiya et al., 2020; Ofoe et al., 2023; Song et al., 2024). Mirzaei et al. (2020) demonstrated that organic fertilization enhanced soil organic matter content and stimulated rhizosphere microbial activity, promoting the complexation of Cd with organic acids and humic substances. This process increased the proportion of HMs fixed in the soil and significantly reduced their bioavailability to plants. However, the transformation processes of HMs in soil and their coupling interactions with plant metabolic pathways remain insufficiently explored. The complexity of these mechanisms stems from differences in soil environmental conditions under organic and conventional cultivation systems, particularly the dynamic regulation of rhizosphere microecosystems. These microorganisms can secrete extracellular polymeric substances (EPS) or organic acids to form complexes with HMs, thereby reducing their mobility and bioavailability (Li et al., 2022). Under organic cultivation, plants often reduce HM accumulation in aerial tissues through mechanisms such as metabolic redistribution, enhanced antioxidant enzyme activity, and the synthesis of metal chelators (Ben Abdallah et al., 2022). These adaptive processes involve the coordinated regulation of multiple metabolic pathways, including glutathione metabolism, metallothionein expression, photosynthesis, and antioxidant defense systems (Kumar et al., 2021). Although previous studies have revealed associations between certain metabolic pathways and HM accumulation, most research has focused on growth cycles spanning 2–6 years (Feszterová et al., 2021; Mirzaei et al., 2020; Zhang et al., 2020a). However, a thorough understanding of the molecular adaptive mechanisms under long-term organic cultivation remains lacking. Furthermore, organic cultivation systems require not only the optimization of field management practices but also a deeper investigation into the potential impacts of surrounding environments on crop growth and HM accumulation. Farmlands near transportation corridors or industrial areas may be subjected to atmospheric deposition of HMs, thereby compromising crop quality and safety (Ahmad et al., 2022). To address this, a comprehensive assessment of regional environmental stressors within organic cultivation systems is essential. Transcriptomic data can be leveraged to uncover patterns of differential gene expression, identify functional genes associated with HM uptake, translocation, and metabolism, and construct their regulatory networks (Yang et al., 2020; Zhao et al., 2021). Such approaches not only enhance our understanding of how different cultivation systems influence the dynamics of HM accumulation in wheat but also provide critical theoretical support for developing precision agricultural management and soil remediation strategies.

The objective of this study is to assess the levels of HM exposure in wheat in Jiyuan (Jy), Yuzhou (Yz) (Henan Province, China) and Camerino (Cam), Jesi (Js) (Marche region, Italy), and to compare the potential impacts of Org and Conv on HM accumulation in the Js experimental field using chemometric methods. The research primarily focuses on the following aspects: 1) analyzing the soil pollution characteristics in different regions and their impact on wheat HM absorption and translocation, and comparing the migration characteristics of soil HMs to wheat in two regions from China and Italy; 2) Investigating the spatiotemporal distribution and dynamic translocation patterns of various HMs in wheat across four key growth stages—tillering (TS), jointing (JS), filling (FS), and maturity (MS)—and among different plant organs, in order to elucidate how HMs are redistributed within the wheat over time; 3) exploring the regulatory effects of Org and Conv cultivation practices on wheat HM accumulation and translocation characteristics. Through a systematic investigation of the effects of regional differences and cultivation practices on HM accumulation in wheat, this study provides novel insights and valuable data to support future research and inform policy development.

2. Materials and methods

2.1. Description of study area

Henan Province is a major wheat-producing region in China, playing a crucial role in ensuring food security both in China and globally, with Jy and Yz located within the province. Jy is rich in mineral resources and has developed into an important industrial center with a comprehensive mining industrial system encompassing mining, smelting, and mineral product processing. Jy, recognized as a key lead processing center in China, is influenced by a continental monsoon climate, experiencing northwest winds in winter, southeast winds in summer, an average annual temperature of 14.3℃, and average annual precipitation of 600–700 mm.The experimental field (cinnamon soil) (35°08’22" N; 112°31’34" E), located near a lead smelting plant, is marked by the blue dashed line (Fig. 1a). Yz, known as the "Capital of Ceramics" and "Hometown of Medicine," is rich in resources. The average annual temperature of Yz is 14.7°C, with an average annual precipitation of 650 mm. The blue dashed line in Fig. 1b indicates the area with cement and machinery processing plants. The selected experimental field (cinnamon soils) is located near Xiaoji Village in Yuzhou (34°13’05" N, 113°33’24" E) (Fig. 1b).

Js and Cam are located in the Marche region of Italy. Js has a typical Mediterranean climate, with an average annual temperature of 13.6°C and average annual precipitation of 780 mm. Strong winds from the north and northwest prevail in the region. The blue dashed line in Fig. 1c marks the industrial area, while the red dashed line represents the railway. Wheat is planted in the experimental field (silty-clay loam texture interspersed with a significant amount of pebbles and cobblestones) of the Agenzia per l’Innovazione nel Settore Agroalimentare e della Pesca “Marche Agricoltura Pesca” (AMAP) in Jesi (43°32’03" N; 13°16’37" E) (Fig. 1c). The research area in Cam is characterized by marl sand dunes towards the internal slope (Fig. 1d). The climate is also Mediterranean, with average annual precipitation ranging from 700 to 900 mm. This area (calcareous clay loams) is far from industrial zones and is considered an unpolluted natural environment area (43°08’34" N; 13°03’07" E) (Fig. 1d).

2.2. Materials and experimental design

These study sites were chosen based on their significant differences in agricultural practices and soil pollution backgrounds, enabling the investigation of wheat’s HM absorption and translocation patterns

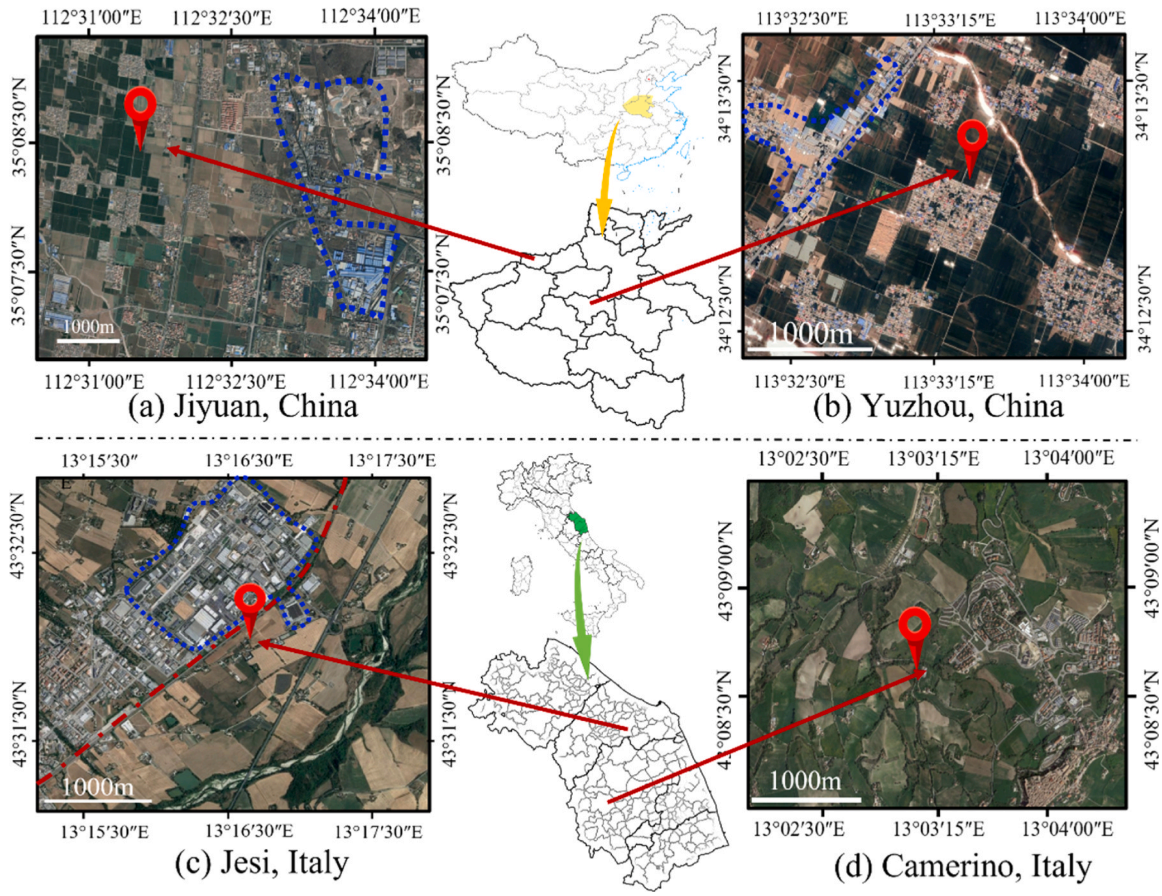

Fig. 1. Location of study area. Note: Blu lines encircle industrial settlements, and Red circles indicate the sampled places. The dashed red line in (c) indicates the railway.

under different environmental conditions and exploring the integrated regulatory effects of cultivation systems on HM accumulation. To further investigate the impact of different cultivation systems on wheat HM accumulation, experimental research was conducted at AMAP experimental field in Js, Italy. AMAP, established in 2004, has functioned as a dedicated organic cultivation trial site, maintaining strictly regulated agricultural practices and environmental conditions. The experimental plots were divided into three standard plots of 7 m $\times$ 1.5 m each. The experimental design included three treatment groups: Org (Organic cultivation, no railway influence), where no synthetic fertilizers or pesticides were used, and the type and quantity of substances applied were strictly controlled. Soil management and cultivation practices were consistent, and the organic plots were located away from railway environments to avoid the potential impact of railway-related HM pollution on the soil. Conv (Conventional cultivation) treatment involved conventional agricultural management, including the use of synthetic fertilizers and pesticides. Org-Rail (Organic cultivation, near railway environment) also followed organic farming regulations, but the plots were located 50 m from the railway. The specific management practices for organic and conventional farming systems are detailed in Table S1. Organic farming strictly adheres to EU organic farming regulations (EC 834/2007 and 889/2008), prohibiting the use of synthetic fertilizers and pesticides, while conventional farming employs routine fertilizers and pesticides. To analyze the dynamic changes in wheat HM absorption and translocation during different growth stages (TS, JS, FS, MS). Graphical flowchart of the study design and objectives is shown in Fig. S1.

2.3. *Preparation of samples*

2.3.1. *Sample collection*

Samples were collected at MS from Yz (Ma 25, 2021), Jy (Ma 23, 2021), Cam (June 21, 2021), and Js (June 14, 2021) for the analysis of environmental impacts in different regions. Additionally, samples were collected from the AMAP experimental field at the Js in Italy at various wheat growth stages (TS, February 7, 2022; JS, April 28, 2022; FS, May 28, 2022; MS, June 14, 2022) to study the effects of different cultivation systems on wheat growth. Rhizosphere soil samples (the soil layer directly influenced by root exudates and associated microorganisms) were collected from the plow layer (0–20 cm). Random sampling was employed, with multiple points set within each study area to obtain representative composite samples while minimizing site-specific bias. Clean polyethylene tools and gloves were used throughout to prevent cross-contamination, and samples were promptly sealed in labeled sterile bags and transported to the laboratory in coolers at 4°C for processing. The root samples of the wheat were washed with tap water to remove surface debris and then rinsed with ultrapure water. After cleaning, the samples were placed in a 105°C drying oven for 30 min to sterilize them, followed by drying at 75°C until a constant weight was achieved. The dried samples were ground to a uniform fine powder using an agate mortar and stored in self-sealing bags for subsequent analysis. Soil samples were air-dried naturally, sieved through a 100-mesh sieve to remove impurities, and then stored in self-sealing bags for later analysis.

2.3.2. Chemical analyses

Dried wheat tissue and soil samples were digested using an Ultra-wave Microwave system (UltraWave™, Milestone, Italy) with a digestion solution containing ultrapure HNO_3 (65 %) and HCl (37 %) (4:1) at 230 ℃ for 1 h (Fattobene et al., 2024). For all the analyses, each sample was replicated three times. The element contents were determined by ICP–OES (ThermoFisher, ICAP™PRO X ICP–OES). For quality control, blank samples were analyzed, and reference materials for soil (GBW07454) and plants (GBW10046) certified by national authorities were used. The recovery rates of elements range between 85.0 % and 115.0 %.

2.4. Pollution assessment, contamination level assessment

2.4.1. Geoaccumulation index

The geoaccumulation index can be used to quantitatively study the pollution level of HM elements in sediments and other materials (Ochiagha et al., 2020). It can not only reflect the impact of natural geological activities on background values but also fully consider the impact of human activities on HM pollution. Its calculation formula is:

$$I_{ngeo} = \log_2\left(\frac{C_n}{1.5 \times B_n}\right) \quad (1)$$

In Eq. (1) C_n represents the concentration of the *n-th* HM in sampled soil and B_n represents its geochemical background value in soil. The background values of the investigated soil elements are in (Table 1) that summarize the literature values, Italy (Bini et al., 1988) and Henan (Cr, Mn, Ni, Cu, Zn, As, Cd, and Pb (Shao and Zhou, 1998), Al (Wei et al., 1991). The coefficient of variation 1.5 is obtained by taking into account the changes in background values that may be caused by rock differences and is used to characterize other effects such as rock texture and sedimentation characteristics (Ochiagha et al., 2020). On the basis of the $I_{n\,geo}$ value ($I_{geo} \le 0$: uncontaminated; $0 < I_{geo} \le 1$: uncontaminated to moderately contaminated, etc.), the contamination status of the HM is classified into seven grades (Table S3).

2.4.2. Heavy metal bioaccumulation factor

Soil quality and HM mobility control the content and amount of HMs taken up by plants (Guo et al., 2022). The bioaccumulation factor (BAF) quantifies the possible transfer of HMs from different types of soils to cultivated wheat tissues.

$$BAF_i = \frac{C_i}{C_{Soil}} \times 100\% \quad (2)$$

Here, C_i is the content of the HMs at the i growth stages (the tillering, jointing, filling, and maturity stages). while C_{Soil} denotes the concentration of the corresponding element in the soil. Soil concentrations were calculated as the average of all samples collected throughout the year from the same locations as wheat sampling. A higher BAF value indicates a stronger ability of wheat tissues to accumulate HMs.

2.5. RNA-Seq analysis

Total RNA was isolated from wheat leaf tissues using the TRIzol® Reagent following the manufacturer's instructions. RNA concentration and purity were determined using a ND-2000 (NanoDrop Technologies), and RNA integrity was evaluated with an Agilent 5300 Bioanalyzer. RNA samples with a total yield ≥ 0.5 μg, concentration ≥ 20 ng/μL, and RNA Quality Number (RQN) > 4.5. RNA-seq libraries were prepared and subsequently sequenced on the Illumina HiSeq™ platform to generate high-throughput transcriptomic data. Differential expressed genes (DEGs) in different parts of leaves between the organic and conventional cultivations were identified by DEGseqand Kyoto Encyclopedia of Genes and Genomes (KEGG) database by the free online platform of Majorbio (www.majorbio.com).

2.6. Statistical analysis

Statistical analyses were performed using the IBM SPSS software package version 20.0. The significance of differences was evaluated using one-way analysis of variance (ANOVA), followed by a least significant difference (LSD) post-hoc test. A significance level of 5 % ($P < 0.05$) was used for all statistical analyses. To analyze the relationships between metals (and metalloids) in the samples, principal component analysis (PCA) was conducted using Unscrambler X 10.2 (CAMO, Oslo, Norway). Graphical representations of the data were created using OriginPro 2018 (OriginLab Corporation, Northampton, MA, USA).

3. Results and discussion

3.1. Concentrations of HMs in soil and wheat as affected by different regions

A clear difference in the spatial distribution of HMs between the investigated Italian and Chinese agricultural soils was observed (Fig. 2a). Among the analyzed elements, Al (938.77–52,519.73 mg·kg^{-1}) and Mn (23.93–998.02 mg·kg^{-1}) were the most abundant. In the Jy region of China, Pb (115.43 mg·kg^{-1}) and Cd (2.50 mg·kg^{-1}) concentrations substantially exceeded the background values for Henan Province (Shao and Zhou, 1998), indicating potential anthropogenic enrichment. The Cd (0.18 mg·kg^{-1}) content in the soil of Yz slightly exceeds the background value of soil in Henan Province. The Al (52519.73 mg·kg^{-1}) content in Js soil far exceeds the background value of Italian soil, and the Cu (37.11 mg·kg^{-1}) content in soil is higher than the background value of Italian soil (Table 1) (Bini et al., 1988). The Al content is exceptionally high and is widely recognized as a major phytotoxic factor that inhibits plant germination and root growth by disrupting cellular structures, ion homeostasis, and antioxidant defense systems (Fang et al., 2020; Rahman et al., 2018). Through the evaluation of soil HM pollution by I_{geo}, the Cd and Pb I_{geo} values in Jy soil were as high as 4.68 and 1.82, while in Yz, the I_{geo} value of Cd was 0.84. The Al element I_{geo} value in the soil of Js region in Italy was as high as 2.63, and the Cu element I_{geo} value was 0.04 (Table 1). It was also found that the types of soil HMs pollution at the Chinese and Italian sites differed. The measured pH values of Italian soils is higher than in Chinese ones: Ca (8.64)>Js (8.43)>Yz (7.87)>Jy (7.75).

Hereafter there are the considerations about the HM composition in

Table 1
Evaluation of soil microelement contamination level.

		Cr	*Mn*	*Ni*	*Cu*	*Zn*	*As*	*Cd*	*Pb*	*Al*	*pH*
Background values of microelement in Italian soil (mg•kg^{-1}) (Bini et al., 1988)		*95*	*873*	*50*	*24*	*68*	*41*	*0.44*	*26*	*5660*	
I_{geo}	Camerino	−6.13	−5.77	−3.37	−2.75	−1.74	−4.78	−2.28	−4.66	−3.18	8.64
	Jesi	−0.84	−0.39	−0.35	0.04	−0.32	−4.6	−1.73	−1.06	2.63	8.43
Background values of microelement in Henan soil (Shao and Zhou, 1998) (Al, China (Wei et al., 1991)) (mg•kg^{-1})		*62.5*	*570*	*27.4*	*20*	*61.9*	*9.4*	*0.065*	*21.8*	*3487*	
I_{geo}	Yuzhou	−0.58	−1.13	−1.75	−1.96	−1.27	−0.64	0.84	−1.97	−1.14	7.87
	Jiyuan	−0.57	−0.53	−0.56	−0.44	−0.46	−0.25	4.68	1.82	−0.59	7.75

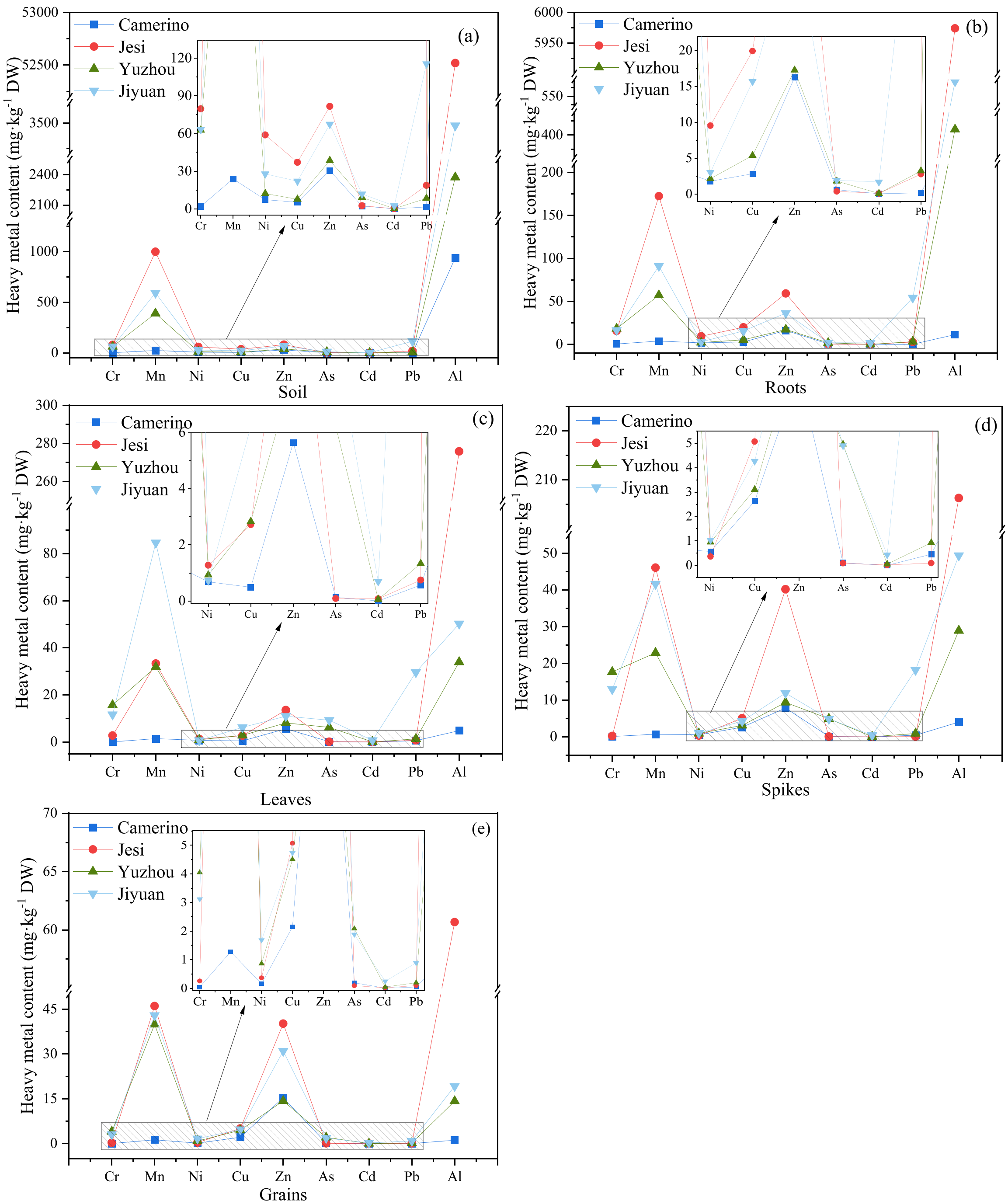

Fig. 2. Heavy metal content ($mg \cdot kg^{-1}$ DW) in soil (a), roots (b), leaves (c), spikes (d), and grains (e) in different regions (Camerino, Jesi, Yuzhou, and Jiyuan). Notes: The shaded area indicates elements with relatively low concentrations, which are enlarged in the inset to improve visibility. The black arrow indicates the corresponding magnified view.

various tissues (roots, leaves, spikes, grains) in the different areas of China and Italy. The HM content ($mg \cdot kg^{-1}$ DW) in wheat grains in the Cam region is lower (Fig. 2) than in the other investigated areas. The distribution of HM content in wheat tissues exhibits a distinctive pattern leaning towards the roots. The hierarchical order of HM accumulation in various tissues is as follows: roots (Fig. 2b) > leaves (Fig. 2c) > spikes (Fig. 2d) > grains (Fig. 2e). In Js wheat grains, there is a notable elevation in the levels of Zn (40.19 $mg \cdot kg^{-1}$) and Al (60.67 $mg \cdot kg^{-1}$) (Fig. 2e). Conversely, the grains of Jy wheat showcase elevated contents of Pb (0.89 $mg \cdot kg^{-1}$), Cd (0.25 $mg \cdot kg^{-1}$), and Ni (1.69 $mg \cdot kg^{-1}$) (Fig. 2e). Relatively high content of Cr (4.05 $mg \cdot kg^{-1}$) and As (2.08 $mg \cdot kg^{-1}$) are present in Yz grain. The different HM BAF indicate that Cr, Ni, and As are more readily translocated to grains in the Chinese wheat compared to the Italian wheat (Table S3). This may be related to the pH of the soil because the soil pH values in Jy (7.75) and Yz (7.87) were lower than in Italian samples (Table 1). Low soil pH has a substantial influence on HM activity, as pH is one of the most important parameters influencing ion forms and solubility in soil (Ali et al., 2020; Zhao et al., 2007). The HM elements Cu and Zn in Chinese and Italian wheat grains are more easily transported to the grains compared to other elements and are enriched in the grains (Table S3). The experimental area exhibits varying degrees of HM pollution, encompassing a diverse range of pollution scenarios. This provides a rich research background, aiding in a comprehensive comparison of wheat's accumulation differences for various HMs. The soil HM content is at a relatively low level in the Cam region, that is far away from industrial enterprise activities, indicating once again that human activities have a strong correlation with soil HMs (Li et al., 2020). The findings indicate that the three sites, Jy, Yz, and Js, factories and metal smelters of varying scales in proximity to wheat cultivation areas and the soil in Jy is primarily contaminated with a combination of Pb and Cd, with wheat grains in Jy exceeding the maximum allowable limit for cereal grain (USEPA, 2012). This could be attributed to the presence of significant metal smelting factories in the Jy region (Liu et al., 2020). In Yz, soil contamination is predominantly due to Cd, possibly linked to the presence of refractory material and metal processing factories in the study area (Hao et al., 2022). This observation further supports the notion that HM content in both soil and wheat samples at different sampling points around pollution sources tends to exhibit greater fluctuations (Zheng et al., 2023).

3.2. The relationship of heavy metals in soils and wheat

Principal Component Analysis (PCA) was applied, after autoscaling of the element contents, to all samples including Soil, Root, Leaves, Spikes, Grains. The investigation reveals significant disparities in soil HM pollution levels between Jy and Js. Principal components PC1 and PC2, which collectively account for 88.1 % of the total variance, provide valuable insights (Fig. 3a). The PCA results emphasize the unique signatures of HM content in each tissue (PC1 direction), shedding light on the diverse impact of local environmental factors on wheat samples from different regions (PC2 axis) (Fig. 3a). Remarkably, each tissue exhibited a distinctive clustering pattern based on the combined HM content. The outcomes of the analysis underline that the Cam samples cluster more densely than those from other regions that is coherent with the lower difference of values measured on the matters here sampled. Wheat roots and soil exhibit a larger dispersion than other wheat tissues implying that, after uptake, HMs mainly accumulate in the roots. The roots act as a barrier, hindering the transfer of HMs to aboveground parts to some extent (Shahid et al., 2017). Itfurther elucidate that Jy soil is predominantly influenced by HMs such as As, Cd, and Pb, while Js soil is primarily impacted by Al, Ni, Zn, Cu, and Mn (Fig. 3a). This divergence may be attributed to distinct pollution sources in the two areas, leading to varying types of HM pollution in the soil.

A Spearman rank correlation average linkage hierarchical clustering analysis using 9 HMs shows (Fig. 3b) two well established groups of the Italian samples, however, the group of Js samples joins, at low distance, the grain samples of both Jy and Yz. The group of Ca samples is well differentiated from the others. Remarkably, the results of cluster analysis align with the findings from principal component analysis, providing a consistent prioritization of various HM pollution sources. Notably, Jy, Yz, and Js exhibit a stronger similarity (Fig. 3b), indicative of a shared pattern in HM pollution. Furthermore, our investigation revealed a discernible level of HM pollution in these three areas. This diversity not only provides potential for an in-depth study of wheat's adaptability and response mechanisms to different HMs but also, through comparing metal pollution conditions in different regions, allows for a deeper understanding of specific metals' impact on wheat (Mor et al., 2022; Zou et al., 2021). Specifically, there is a significant correlation in the HM contents of grains from Jy and Yz. This underscores the interconnected nature of HM pollution in these regions and emphasizes the need for targeted interventions and mitigation strategies to address the shared challenges across Jy, Yz, and Js.

Soil samples in the research area show minimal contamination by Cr, Mn, Ni, Zn, and As (Table 1). Comparing the BAF of different HMs in

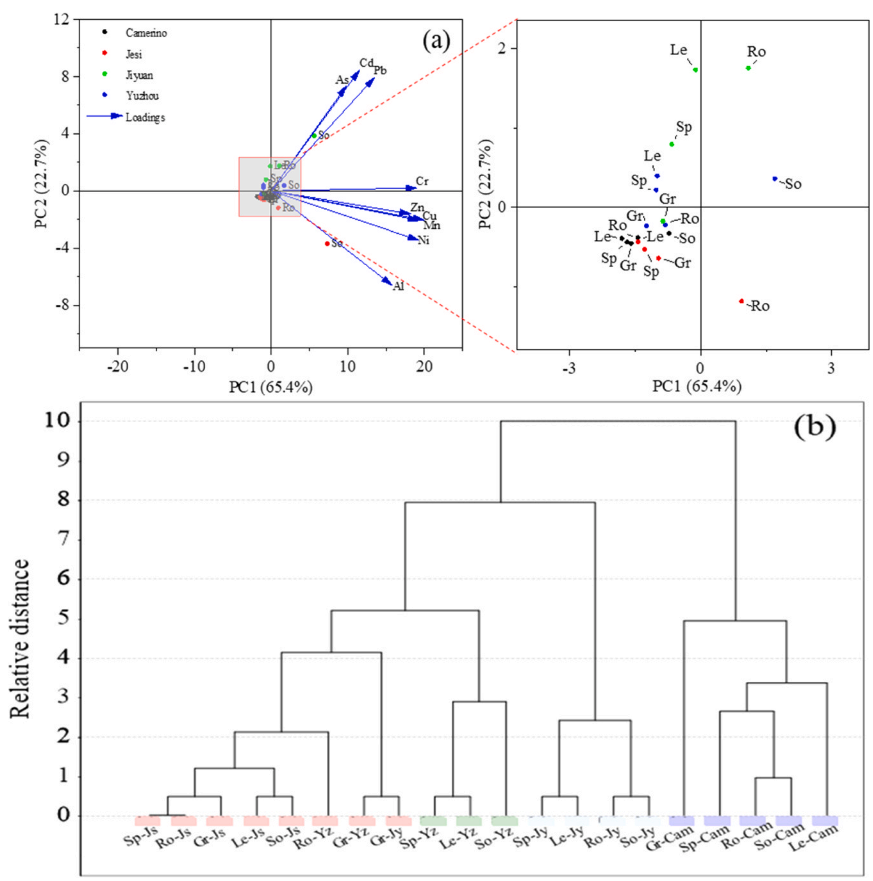

Fig. 3. (a) Bi-plot projection of the first two components computed in different regions is related to the wheat tissues and soil heavy metal content; (b) Heavy metal contents in samples (roots, leaves, spikes, grains, soil) from different regions were analyzed using Spearman rank correlation average linkage clustering analysis. Abbreviation notes: Camerino-Cam, Yuzhou-Yz, Jesi-Js, Jiyuan-Jy; Soil-So, Root-Ro, Leaves-Le, Spikes-Sp, Grains-Gr.

various wheat tissues, it was observed that in the Cam region, the BAF of Cr (4.89 %) and Cu (49.15 %) in the spike are higher than that in wheat shoots (leaves, grains), indicating a tendency for Cr and Cu to accumulate in the spikes (Table S3). Similar results are found in the Js region, where the BAF of Cr (3.50 %) and Mn (4.66 %) in wheat spikes are relatively high (Table S3). In Yz (28.24 %) and Jy (20.56 %), the BAF of Cr in spikes are higher compared to other tissues like leaves and grains (Table S3). In summary, it found that Cr tends to accumulate more in the upper parts, particularly in the spikes. Pb, on the other hand, tends to accumulate more in leaves. Additionally, when comparing the BAF of different HMs between the shoots and roots, we observed significant differences for Cd and Al. This suggests that Cd and Al are more prone to accumulate in the roots and, to some extent, restrict their transfer to the shoots (Poschenrieder et al., 2008; Van Der Vliet et al., 2007). Through the comparative analysis between Chinese and Italian samples, it was observed that the content of Cu, Zn, and Al in the leaves, spikes, and grains of Js wheat is higher than that in wheat tissues from other regions. The Geo Accumulation Index was employed to assess metal enrichment in soil, with Cu (I_{geo}=0.04) and Al (I_{geo}=2.63) indicating the phenomenon of HM enrichment in wheat. However, the ability of Zn to accumulate in grains surpasses that of Cu and Al but it is known that OsHMA2 and OsZIP3 proteins play a role in the preferential distribution of Zn to the grain in the node (Cai et al., 2019; Xia et al., 2023). Unlike other HMs members, OsHMA2 functions as an inland transporter for Zn, prioritizing its transport to the grains due to its long C-terminal region (Lei et al., 2021; Yamaji et al., 2013).

3.3. *Contents of HMs in soil and wheat as affected by different cultivation methods*

After 17 years (2005–2022) of continuous implementation of Org and Conv fertilization treatments, a significant reduction in soil HM contents was observed. The average contents of soil HMs under Conv were Cr (89.14 ± 0.38 $mg\cdot kg^{-1}$), Mn (1068.42 ± 4.53 $mg\cdot kg^{-1}$), Ni (62.00 ± 0.24 $mg\cdot kg^{-1}$), Cu (33.28 ± 1.50 $mg\cdot kg^{-1}$), Zn (89.51 ± 3.17 $mg\cdot kg^{-1}$), As (3.15 ± 0.06 $mg\cdot kg^{-1}$), Cd (0.23 ± 0.01 $mg\cdot kg^{-1}$), Pb (22.34 ± 0.86 $mg\cdot kg^{-1}$), and Al (52160.38 ± 1036.68 $mg\cdot kg^{-1}$) (Fig. 4a). In contrast, under Org, the average contents of these HMs decreased by Cr (28.99 %), Mn (22.70 %), Ni (28.82 %), Cu (11.16 %), Zn (17.44 %), As (31.78 %), Cd (35.39 %), Pb (14.97 %), and Al (5.75 %) (Fig. 4a). This significant reduction trend indicates that Org effectively reduced HM accumulation in the soil by minimizing or avoiding the use of synthetic fertilizers and pesticides. Moreover, after 17 years of different cultivation practices, the average concentration of HMs in the soil under Org was 21.89 % lower than under Conv, with an annual reduction of 1.29 %. However, the concentration of HMs in Org-rail showed an increase, with Cr, Ni, and Cd being 24.78 %, 22.45 %, and 29.27 % higher than in the Org, respectively. This phenomenon suggests that environmental pollution sources near the railway significantly affected the accumulation of Cr, Ni, and Cd in soils, likely originating from the HM particles emitted during railway transport. This finding underscores the important role of environmental background in the accumulation of HMs under Org, and highlights the need for enhanced environmental monitoring and pollution control in agricultural areas near railways. This will ensure soil health and the sustainable development of agriculture while promoting organic cultivation.

To further understand the impact of different cultivation systems on soil HMs, we analyzed the relationship between soil pH and HM elements, as well as the correlations between different HMs under various cultivation practices. A high correlation (greater than 0.8, $P < 0.05$) was observed between Cr, Ni, Zn, Cu, As, Cd, Pb, and Al in the soil (Fig. 4b), suggesting that elements showing substantial correlations likely have a common origin or comparable deposition processes (Hao et al., 2024). This indicates that these eight HM elements may share a common source and be simultaneously influenced by various sources. In contrast, Mn showed a negative correlation with other elements and a significant negative correlation with Al. The results also revealed that pH values were positively correlated with most HM contents, significantly affecting the geochemical behavior of HMs in the soil (Liu et al., 2022; Swęd et al., 2022). pH was significantly positively correlated with Cr, Ni, Zn, Cd, and Al (Fig. 4b). However, the correlation with Cu, As, and Pb was lower. Generally, lower pH value tend to increase the solubility and plant uptake of certain HMs, while higher pH values decrease their solubility and bioavailability (Ofoe et al., 2023; Song et al., 2024). The changes in HMs in wheat roots at different growth stages exhibited similar trends (Fig. S2). Org significantly reduced the contents of most HMs in wheat roots, particularly during the TS and JS, where the levels of Cr, Mn, Ni, Cu, Zn, As, Cd, Pb, and Al were all significantly lower compared to the Conv, with reductions ranging from 5.75 % to 54.19 % (Fig. S2). While Org maintained its advantages to some extent, due to HM emissions during railway transportation, Org-Rail showed higher contents of certain HMs (Cr, Mn, Ni, Cu, Zn, As, Cd, Pb, and Al) in the roots compared to the Org. Notably, during the TS and JS, the contents of Cr, Mn, Ni, and Cd significantly increased, with levels rising by

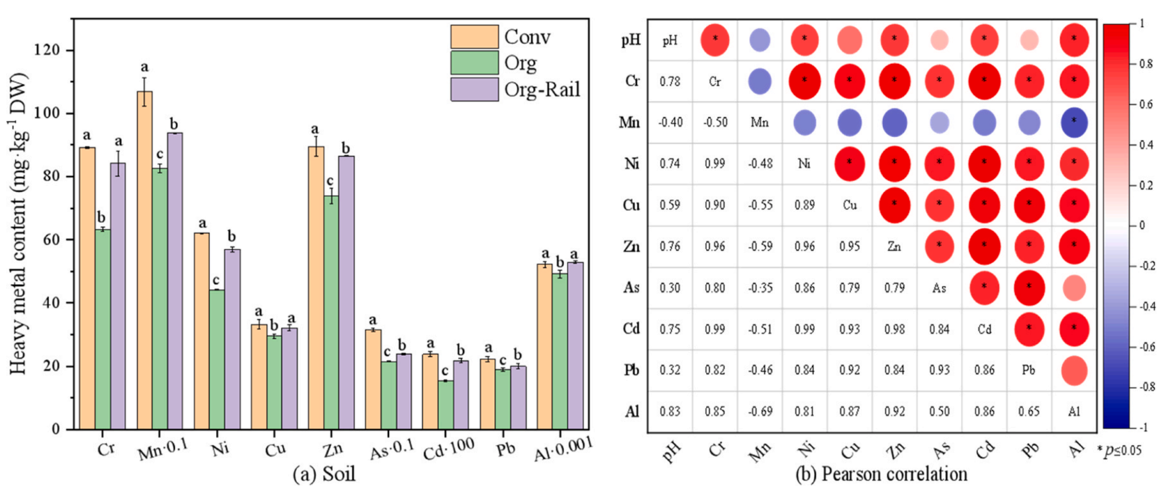

Fig. 4. (a) Heavy metal content ($mg\cdot kg^{-1}$ DW) in rhizosphere soil under different cultivation modes; (b) Pearson correlation coefficient of heavy metals and pH in different cultivation soils. Notes: 0.001, 0.1, 10, and 100 represent a 1000-fold reduction, 10-fold reduction, 10-fold magnification, and 100-fold magnification, respectively, of the corresponding element content; Conv, Org, and Org-Rail represent conventional cultivation, organic cultivation, and organic cultivation near railway environments, respectively, with different letters (a, b, c) in the figure indicating significant differences in heavy metal content among treatments ($P \leq 0.05$); *represents the statistical significance at the $P \leq 0.05$ level.

approximately 15.43–51.21 % (Fig. S2). This suggests that environmental pollution sources near the railway have a significant impact on the accumulation of these HMs in wheat roots. The HM in wheat plants exhibited significant differences across different growth stages and cultivation systems, with organic cultivation demonstrating a sustained advantage in controlling HM accumulation. Specifically, wheat's ability to absorb HMs varied across growth stages, leading to significant differences in the HM content in the wheat straws at different stages (Fig. 5). During the wheat straws growth cycle, the HM content was lowest during the JS (Fig. 5b), indicating reduced accumulation activity at this stage. In contrast, Mn, Cd, and Al levels were higher during theTS (Fig. 5a), while Ni, As, and Pb contents peaked during the FS and MS (Figs. 5c, 5d). When comparing cultivation systems, Org effectively reduced the accumulation of most HMs in wheat straws, with the most significant reductions observed during the JS (Fig. 5b) and FS (Fig. 5c). During the JS Org reduced Cr and As contents in wheat straws by 42.43 % and 68.89 %, respectively. In the FS, HMs such as Ni (26.81 %), Mn (20.13 %), and Al (47.03 %) also showed significant reductions (Fig. 5c). Furthermore, during the MS, the reductions in Cu (54.89 %), Zn (50.05 %), and Cd (42.73 %) were the greatest (Fig. 5d). These results highlight the continuous advantage of Org in controlling HM accumulation during various growth stages. However, during the JS and TS, the Zn content in straws under Org was significantly higher than under Conv (Figs. 5a, 5b). This phenomenon may be attributed to the application of organic fertilizers, which increased the solubility of Zn in the soil, thereby enhancing the plant's absorption of Zn (Kasiviswanathan et al., 2024). Additionally, Org-Rail exhibited significant increases in the contents of several HMs, especially Cr, Ni, Cu, As, Cd, and Pb, indicating that HM emissions from railway transportation strongly impacted organic farming. During the JS and FS, the HM particles emitted by the railway were more readily absorbed by the straw, resulting in a significant increase in the contents of these HMs in the Org-Rail. Based on the trends of HM changes in wheat straws during different stages, the variations in Pb and Cd were similar during the TS and JS. In the FS, Pb content significantly increased, while Cd contents were significantly reduced in both the Org and Org-Rail (with Cd at 0.025 $mg{\cdot}kg^{-1}$ and Pb at 1.61 $mg{\cdot}kg^{-1}$ in the Org). This phenomenon may be attributed to the synergistic absorption of Pb and Cd at early stages. However, in the later stages, limited competition for adsorption sites in the straws led to the rapid accumulation of Pb, which suppressed Cd absorption (He et al., 2020; Zhang et al., 2020b).

Under different cultivation methods, the HM content in wheat grains from Conv was significantly higher than that from Org, except for Ni (Fig. 6). The differences in Cr, Cd, and Pb contents were most pronounced, with Org reducing Cr, Cd, and Pb in wheat grains by 45.19 %, 40.21 %, and 43.93 %, respectively (Fig. 6b). The application of organic fertilizers increased the organic matter content in the soil, which can form complexes with HMs, reducing their bioavailability in the soil and inhibiting plant absorption of these metals (Li et al., 2024; Hu et al., 2021). During the MS, the content of Mn, Ni, Cu, Zn, As, and Al in wheat grains decreased by 7.87 %, 0.87 %, 27.00 %, 16.66 %, 11.29 %, and 9.33 %, respectively (Fig. 6b). The effect of Org on Ni was not significant, and even during the FS, the Ni content in wheat grains under Org slightly increased (Fig. 6a). Some components of organic fertilizers may promote the dissolution and migration of Ni, increasing its bioavailability in the soil (Turan, 2022). This might have altered the pathway of Ni absorption by wheat, making its accumulation in the plant less inhibited. In the FS, Org significantly reduced Cd (approximately

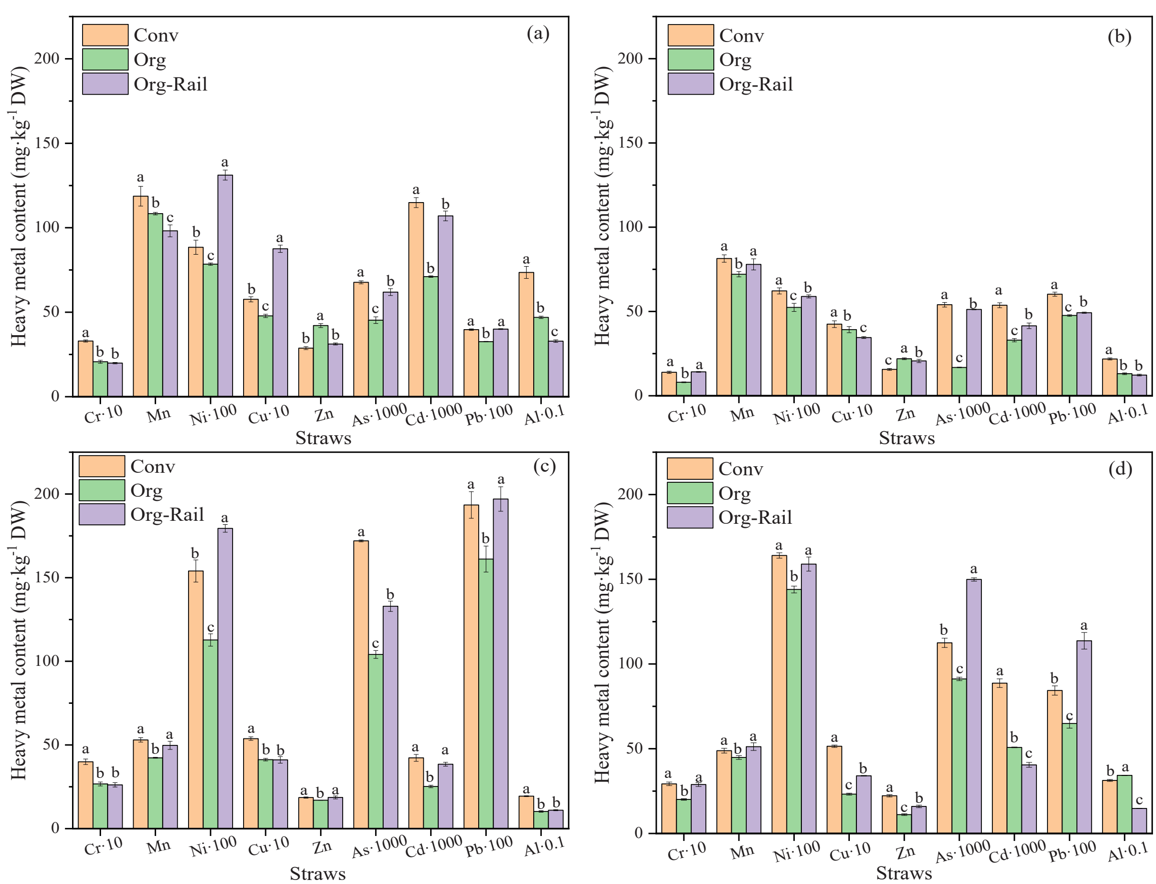

Fig. 5. Heavy metal content ($mg{\cdot}kg^{-1}$ DW) of wheat straws at different stages under different cultivation modes: (a) tillering stage, (b) jointing stage, (c) filling stage, (d) mature stage. Notes: 0.1, 10, 100 and 1000 represent a 10-fold reduction, 10-fold magnification, 100-fold magnification, and 1000-fold magnification, respectively, of the corresponding element content; Conv, Org, and Org-Rail represent conventional cultivation, organic cultivation, and organic cultivation near railway environments, respectively, with different letters (a, b, c) in the figure indicating significant differences in heavy metal content among treatments ($P \leq 0.05$).

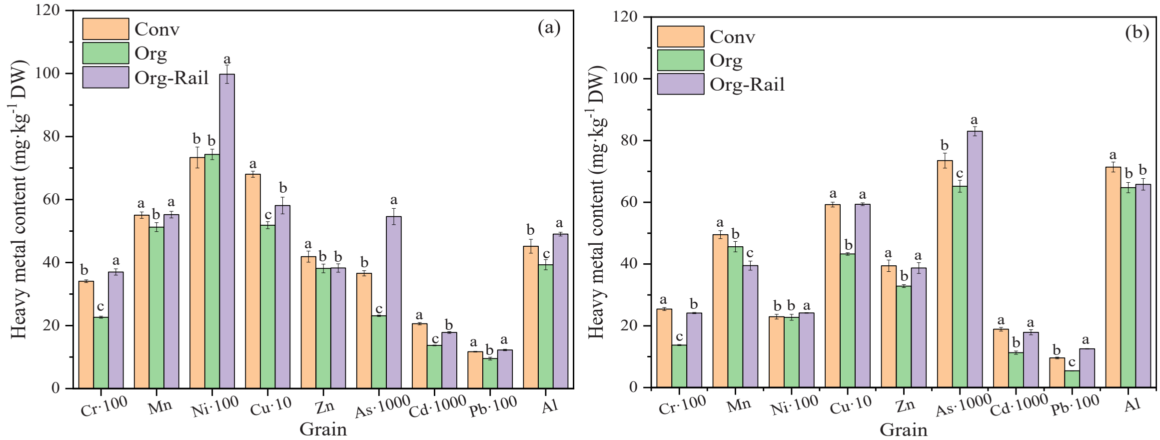

Fig. 6. Heavy metal content (mg·kg^{-1} DW) in wheat grains at different stages under different cultivation modes (a) during grain filling and (b) during mature stage. Notes: 10, 100 and 1000 represent 10-fold magnification, 100-fold magnification, and 1000-fold magnification, respectively, of the corresponding element content; Conv, Org, and Org-Rail represent conventional cultivation, organic cultivation, and organic cultivation near railway environments, respectively, with different letters (a, b, c) in the figure indicating significant differences in heavy metal content among treatments ($P \leq 0.05$).

33.50 %) and Zn (approximately 8.92 %) in wheat grains. During the MS, Org further reduced Cd (approximately 40.21 %) and Zn (approximately 16.66 %) compared to Conv. Organic farming may promote the growth of specific microorganisms, which in turn influence the bioavailability of HMs through processes such as chelation, precipitation, or microbial fixation (Li et al., 2024; Hu et al., 2021). These interactions may limit wheat competition for uptake, ultimately reducing the accumulation of HMs in wheat tissues. The complex interactions between organic amendments, microbial activity, and metal bioavailability may contribute to more controllable metal uptake mechanisms, thereby mitigating the harmful effects of HM pollution on crops. Compared to Org, the Org-Rail showed a significant increase in the content of most HMs. The average HM content in wheat grains under Org-Rail was 21.35 % higher than in the Org (Fig. 6b). During the FS, the contents of Cr, Ni, As, and Pb were higher in the Org-Rail than in the Org. In the MS, the contents of Cr, Cu, Cd, and Pb saw a substantial increase, rising by 42.98 %, 27.14 %, 36.87 %, and 56.73 %, respectively (Fig. 6b). This highlights the significant impact of the railway environment on HM contamination in wheat grains. HM pollutants emitted during railway transport enter the agricultural ecosystem through air deposition, soil contamination, and other pathways. Since the aerial parts of wheat can absorb HMs directly from the dust through stomata (Ma et al., 2021), this significantly affected the effectiveness of organic cultivation in controlling HM accumulation.

3.4. The relationship of HMs in soils and wheat under different cultivation modes

Compared to conventional agriculture, organic farming allows for more limited use of chemical pesticides and synthetic fertilizers (Chabert and Sarthou, 2020). After automatic scaling of variables, samples from the Js experimental field were subjected to PCA. Through the Bi-plot, the effects of different factors on sample characteristics and the relationships between variables were visually observed (Fig. 7). PCA showed that the first principal component (PC1, 88.0 %) and the second principal component (PC2, 8.2 %) explained most of the variance in the data. These two components accounted for 96.2 % of the total variance (Fig. 7). PCA revealed that the soil samples were distinctly separated from other sample materials along the PC1 direction, effectively distinguishing between Conv, Org, and Org-Rail cultivation systems. The other samples clustered based on material, primarily due to PC2, with weaker differentiation between organic and conventional samples, and subtle subgroupings based on growth stages. Cd and Zn contributed significantly to PC1 in the positive direction, indicating that these

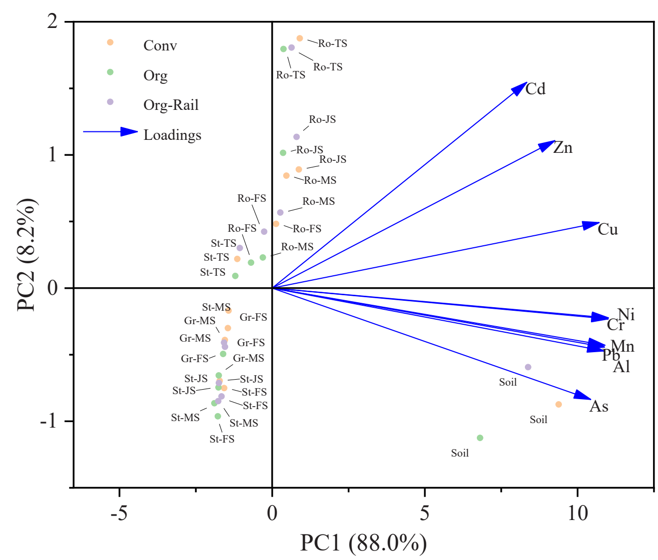

Fig. 7. Bi-plot project of the first two components calculated in different cultivation modes is related to the wheat issue and soil heavy metal content. Notes: The abbreviations in the figure represent the following: Soil (So), Root (Ro), Leaves (Le), Spikes (Sp), Straws (St), and Grains (Gr). The growth stages are Tillering (Ts), Jointing (Js), Filling (Fs), and Mature (Ms). The yellow points represent the conventional cultivation system (Conv), the green points indicate the organic cultivation system (Org), and the purple points denote the organic cultivation system near railway environments (Org-Rail).

elements primarily drove the variation in PC1. Cu also showed a notable positive contribution, although its influence was slightly less than that of Cd and Zn. As, Ni, Pb, Mn, and Al contributed negatively to PC2, reflecting the differences in the accumulation of these elements in wheat and its environment. It was found that the wheat roots were most influenced by Cd, Zn, and Cu (Fig. 7). The HM content in wheat roots during the TS showed a strong correlation, while the Pb content in wheat grains during the FS and JS showed a significant correlation with the Pb content in the wheat straws. Samples from the Org-Rail in the Bi-plot were closer to the Conv samples (Fig. 7), indicating that due to railway pollution, Org-Rail exhibited increased similarities with Conv in terms of HM content, weakening the low HM advantage that Org should have had. Therefore, green buffer zones, such as vegetation barriers, should be established between railways and agricultural areas to reduce the diffusion and deposition of HM particles. To further understand the accumulation of HMs in different stages and wheat tissues (roots, straws, and grains), we performed PCA on the BAF of different HMs and explored their response characteristics during different growth stages of wheat based on the Bi-plot (Fig. S3). PCA analysis revealed that the first principal component (PC1, 75.2 % of total variation) and the second principal component (PC2, 10.8 % of total variation) jointly explained 86.0 % of the data variation, reflecting the distribution and translocation characteristics of HMs under different treatments. There were significant differences in HM enrichment across wheat roots, straws, and grains. Most root samples clustered toward the right along the PC1 axis, indicating that the BAF of HMs in the roots were generally higher. Most HMs were enriched in the roots of wheat. The enrichment of Al and Zn in the roots was particularly prominent during the TS and MS. In the MS, the BAF of Zn in wheat grains was much higher than that of other HMs. Grains and straws exhibited overall lower accumulation levels, as reflected in the negative direction of PC1, but the characteristics differed: grains tended to accumulate Zn and Cu, while straws showed a preference for Cd accumulation. This finding is similar to the results observed for wheat in the two regions in China, reflecting the significant differences in HM translocation characteristics between wheat tissues.

To better understand the impact of different cultivation systems on HMs, and based on previous studies indicating that wheat leaves are the primary contributors to HM accumulation in wheat grains, further focused on the bioinformatics changes in wheat under various cultivation conditions to reveal the mechanistic effects on wheat's physiological and genetic basis. Since wheat leaves play a crucial role in wheat growth, development, immune defense, and metabolic regulation (He et al., 2022; Zhao et al., 2021), transcriptomic analysis of these leaves can help us gain deeper insights into the effects of organic and conventional cultivation on wheat's growth and physiological processes. From the volcano plot, RNA-seq identified 25,102 differentially expressed genes (DEGs) in wheat leaves from organic and conventional cultivation systems, including 12,608 upregulated DEGs and 12,494 downregulated DEGs (Fig. S4). The identification of these DEGs enabled a comprehensive understanding of the impact of Org and Conv on wheat leaves gene expression, allowing for further exploration of the biological significance of these genes. Through KEGG enrichment analysis, the enrichment of these DEGs in various biological pathways was explored (Fig. 8). KEGG enrichment analysis helped understand the biological functions of DEGs in metabolic pathways, signal transduction, immune responses, and more, revealing the mechanisms of how different cultivation systems affect the bioinformatics of wheat leaves (Yang et al., 2020). In wheat Conv, 20 KEGG pathways were significantly altered, including photosynthesis-antenna proteins, valine, leucine, and isoleucine degradation, phenylalanine, tyrosine, and tryptophan biosynthesis, linoleic acid metabolism, peroxisome, and glutathione metabolism (Table S4). Fig. 8a shows that the Glutathione metabolism pathway was less affected, with only 162 genes involved. The Photosynthesis-antenna proteins pathway is a significantly different KEGG pathway in different wheat cultivation. The pathway contains only 55 genes and has a maximum rich factor of 0.56 (Table S4). It is a crucial component of the energy capture and conversion process in photosynthesis. Antenna proteins absorb photons through pigment molecules such as chlorophyll and carotenoids, and transfer the energy in the form of excited states to the reaction centre, initiating the photochemical reactions of photosynthesis (Fan et al., 2019; Feng et al., 2023).

Under Org, wheat may be exposed to more natural stresses (e.g., pests, diseases, weed competition, etc.), which may prompt plants to adjust their photosynthetic antenna protein pathways to enhance adaptation to these stresses. In contrast, Conv reduces these natural stresses through external inputs, which may result in plants differing in the expression of the photosynthetic antenna protein pathway. The analysis of 55 genes in the Photosynthesis-antenna proteins pathway (Fig. 8b) revealed that the expression levels of three gene fragments (TraesCS5B02G322900, TraesCS5A02G322500, TraesCS6B02G122600) differed most significantly. Searching the TrEMBL database found (Source: UniProtKB/TrEMBL; Acc:W5F8Z5), these three genes are closely related to Chlorophyll$_{a\text{-}b}$ binding protein, chloroplastic. They are responsible for capturing and transmitting light energy of chlorophyll molecules during photosynthesis (Chang et al., 2024; Lan et al., 2023). Therefore, the photosynthetic antenna protein pathway has a crucial impact in Org and Conv. This finding emphasizes the regional differences within organic agriculture systems, providing crucial insights for a better understanding of the impact of organic agriculture on the accumulation of trace elements. By studying these differences in greater depth, a better response to varying agricultural management practices can be achieved, allowing for the optimization of agricultural production methods and the enhancement of crop productivity and environmental adaptability.

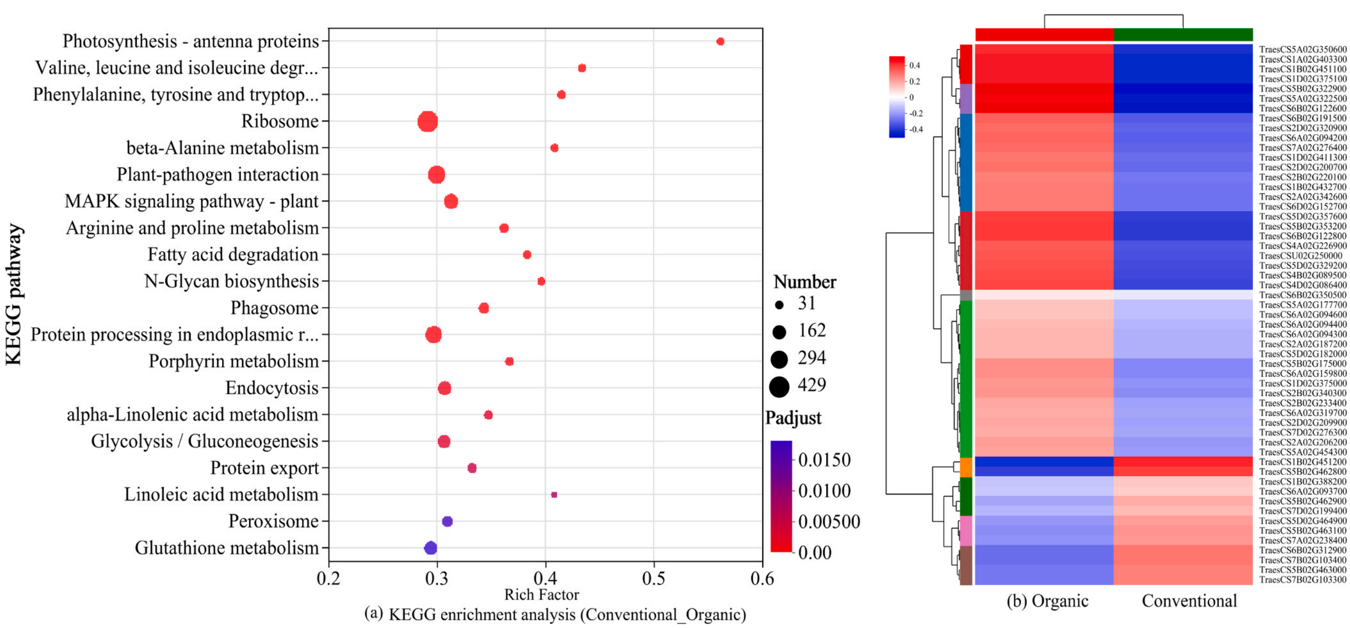

Fig. 8. KEGG enrichment analysis (a), Clustering heatmap analysis (b) of wheat leaf genes under organic and conventional cultivation (The red indicating higher expression and blue indicating lower expression).

4. Conclusion

This study reveals critical insights into HM accumulation patterns in wheat and associated soils at representative sites in China and Italy, highlighting the significant impact of regional human activities on environmental pollution. Compared to Conv, Org reduces the average HM content in soil by 1.29 % annually. The roots showed higher accumulation activity for different HMs during the TS and JS. The grains exhibited the most significant accumulation of Pb and As during the FS and MS. Railway transportation had the most notable effect on the accumulation of Cr, Cu, Cd, and Pb in wheat grains. Different cultivation systems mainly affect the photosynthesis-antenna protein pathway, highlighting a potential biological mechanism underlying observed differences in HM accumulation.

CRediT authorship contribution statement

Mario Berrettoni: Writing – review & editing, Validation, Supervision, Investigation, Conceptualization. **Hongzhong Zhang:** Writing – review & editing, Supervision, Conceptualization. **Paolo Conti:** Supervision, Software, Investigation, Formal analysis. **Silvia Zamponi:** Supervision, Investigation, Formal analysis. **Raffaele Emanuele Russo:** Formal analysis, Data curation. **Elisa Santoni:** Methodology, Investigation. **Martina Fattobene:** Software, Investigation, Data curation. **Chuang Ma:** Supervision, Investigation, Funding acquisition, Formal analysis, Data curation. **Fuyong Liu:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2025.118875.

Data availability

Data will be made available on request.

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