Science of the Total Environment 748 (2020) 142484

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Effect of the irrigation method and genotype on the bioaccumulation of toxic and trace elements in rice

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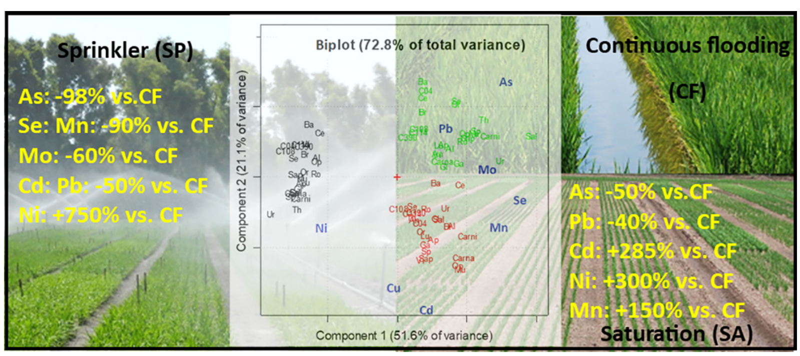
GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 June 2020
Received in revised form 14 August 2020
Accepted 17 September 2020
Available online 24 September 2020

Editor: Charlotte Poschenrieder

Keywords:
Toxic elements
Oligoelements
Arsenic
Cadmium
Lead
Nickel

ABSTRACT

The total concentration of three toxic elements (As, Cd and Pb) and five oligoelements (Cu, Mn, Mo, Ni and Se) has been determined using an original and completely validated ICP-MS method. This was applied to rice grains from 26 different genotypes cultivated in the same soil and irrigated with the same water in three different ways: by the traditional continuous flooding (CF) and by two intermittent methods, the sprinkler irrigation (SP) and the periodical saturation of the soil (SA). The adoption of SP hugely minimizes the average amounts of almost all elements in kernels (−98% for As, −90% for Se and Mn, −60% for Mo, −50% for Cd and Pb), with the only exception of Ni, whose concentration increases the average amount found in the CF rice by 7.5 times. Also SA irrigation is able to reduce the amounts of As, Mo and Pb in kernels but it significantly increases the amounts of Mn, Ni and – mainly - Cd. Also the nature of the genotype determined a wide variability of data within each irrigation method. Genotypes belonging to *Indica* subspecies are the best bioaccumulators of elements in both CF and SP methods and, never, the worst bioaccumulators for any element/irrigation method combination. In the principal component analysis, PC1 can differentiate samples irrigated by SP by those irrigated by CF and SA, whereas PC2 provides differentiation of CF samples by SA samples. When looking at the loading plot Ni is negatively correlated to the majority of the other elements, except Cu and Cd having negative loadings on PC2. These results allow to envisage that a proper combination of the irrigation method and the nature of rice genotype might be a very valuable tool in order to successfully achieve specific objectives of food safety or the attainment of functional properties.

Abbreviations: ICP-MS, inductively coupled plasma – mass spectrometry; CF, continuous flooding irrigation; SA, saturation.

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https://doi.org/10.1016/j.scitotenv.2020.142484
0048-9697/

1. Introduction

Nowadays there is a widespread concern related to the contamination of ecosystems by toxic or potentially toxic elements originated from both natural and anthropic sources (Kabata-Pendias and Mukherjee, 2007). Ingestion represents for humans the main route of intake of these elements whose effects depend not only on the type of element but also on the levels of contamination and on the diet composition (Kabata-Pendias and Mukherjee, 2007). Whereas elements like As, Cd and Pb cause always adverse effects in humans regardless their concentration (Kabata-Pendias and Mukherjee, 2007), oligoelements like selenium (Se), nickel (Ni), copper (Cu), molybdenum (Mo) and manganese (Mn), although essentials for biotic communities, may become harmful as a consequence of an excessive intake (WHO, 1996), because their toxicity threshold is often close to the levels requested to exert their metabolic action.

Rice is the most important cereal for human nutrition. Currently, it is the staple food for more than 60% of mankind, with a daily intake largely up to 0.5 kg per capita in Myanmar, Bangladesh, Vietnam, Cambodia, Indonesia and Laos (FAO, 2002). In the near future, rice production is expected to be even more important due to the rapid demographic increase in those countries where it already provides the highest caloric intake to their inhabitants (United Nations, Department of Economic and Social Affairs, Population Division, 2015). Contaminated paddy fields along with the use of rice genotypes with high tendency of bioaccumulation of toxic elements are the most frequent causes of health risk for these populations. For example, the presence at high concentrations of As and Cd in rice kernels represents a serious issue for the populations of South and South-eastern Asia (Marin et al., 1993; Meharg and Rahman, 2003; Kawada and Suzuki, 1998; Meharg et al., 2009; Tsukahara et al., 2003; Duxbury and Panaullah, 2007). For this reason, Codex Alimentarius (FAO-WHO Codex Alimentarius, 2014; FAO-WHO Codex Alimentarius, 2020), European Union (Commission Regulation (EU), 2015; Commission Regulation (EC), 2006) and Countries like Popular Republic of China (Popular Republic of China, 2005) and Japan (Japan External Trade Organization, 2011) set upper limits in the amounts not only for As and Cd, but also for Pb in rice grain.

The conditioning of the soil with nutrients competing in the absorption process of toxic elements by rice roots (Suda and Makino, 2016; Meharg and Meharg, 2015; Tripathi et al., 2014; Saifullah et al., 2018; Suriyagoda et al., 2018; Sebastian and Prasad, 2013; Li et al., 2017), the organic pharming (Islam et al., 2016; Uraguchi and Fujiwara, 2013), phytoremediation (Suriyagoda et al., 2018; Srivastava et al., 2012; Rizwan et al., 2016) and bioremediation (Li et al., 2017; Rizwan et al., 2016; Upadhyay et al., 2018; Hu et al., 2016) along with the employment of either genetically modified (Sebastian and Prasad, 2013; Li et al., 2017; Uraguchi and Fujiwara, 2013; Srivastava et al., 2012; Upadhyay et al., 2018; Miura et al., 2011; Zhao and Wang, 2019) and selected rice varieties with a low As- (Saifullah et al., 2018; Islam et al., 2016; Mitra et al., 2017) and Cd- (Rizwan et al., 2016; Hu et al., 2016; Grant et al., 2008) bioaccumulation tendency were proved to be successful in the reduction of the concentration of these elements in rice (Suda and Makino, 2016; Meharg and Meharg, 2015; Tripathi et al., 2014; Saifullah et al., 2018; Suriyagoda et al., 2018; Sebastian and Prasad, 2013; Li et al., 2017; Islam et al., 2016; Uraguchi and Fujiwara, 2013; Srivastava et al., 2012; Rizwan et al., 2016; Upadhyay et al., 2018; Hu et al., 2016; Miura et al., 2011; Zhao and Wang, 2019; Mitra et al., 2017; Grant et al., 2008; Bakhat et al., 2017; Chaney et al., 2007).

The nature of irrigation methods influences the bioavailability of As and Cd in soils as well (Honma et al., 2016). While the continuous flooding irrigation, CF (i.e. the worldwide traditional method) is effective in minimizing the Cd concentration in rice grain (Arao et al., 2009; Hu et al., 2013a; Hu et al., 2013b; Moreno-Jiménez et al., 2014; Sun et al., 2014), it may cause a dramatic increase in As concentration (Arao et al., 2009; Hu et al., 2013a; Hu et al., 2013b; Moreno-Jiménez et al., 2014; Sun et al., 2014), reaching concentration levels of mg kg^{-1} units (Meharg and Rahman, 2003). Conversely, an opposite behavior has been often observed adopting alternative irrigation techniques (i.e. the so-called "aerobic", "intermittent" or "oxidized" methods (Honma et al., 2016; Arao et al., 2009; Hu et al., 2013a; Hu et al., 2013b; Moreno-Jiménez et al., 2014; Sun et al., 2014)). However, these methods are often not adequately described, and serious doubts remain about how rice was effectively irrigated (Spanu et al., 2018). Intermittent irrigation methods, like the sprinkler (SP) and the periodical saturation of the soil (SA), have been optimized and used in open field rice cultivations in Sardinia, Italy (Spanu et al., 2018; Spanu et al., 1989; Spanu et al., 2004a; Spanu et al., 2009; Spanu et al., 2012; Spanu et al., 2020). The replacement of CF with SP (Spanu et al., 1989; Spanu et al., 2004a; Spanu et al., 2009), applied over decades on several rice genotypes, has produced along with comparable yields a number of environmental advantages including halved water requirements, minimized number and intensity of treatments against weeds, the avoidance of the soil levelling and the use of specific agricultural machinery (Spanu et al., 2004a). In addition, an outstanding and unprecedented reduction of the average As amount in kernels was observed for the SP irrigation (ca. -98% in 37 different rice genotypes) (Spanu et al., 2012) together with a 20% reduction in the average Cd concentration in kernels of 26 genotypes (Spanu et al., 2018). On the other hand, an extraordinary increase of Cd concentration (between 760 and 1000% of the average values measured for CF) has been measured in rice grain irrigated with SA (Spanu et al., 2018).

Beyond As and Cd, also another toxic element like lead (Pb) may be bioaccumulated in rice. The element is also easily detectable in a large number of other cereals and vegetables (Norton et al., 2015). After As, Pb is ranked second on the Priority List of Hazardous Substances of the US Agency for Toxic Substances and Disease Registry (US Agency for Toxic Substances and Disease Registry (ATSDR), 2017). The major causes of its toxicity are the strong interaction with SH- groups in proteins and the interference with the homeostasis of essential divalent cations. Pb is highly neurotoxic for children, even at very low exposure levels (Canfield et al., 2003), and this has caused the withdrawal of its provisional tolerable weekly intake (PTWI), fixed by Joint FAO/WHO Expert Committee on Food Additives (FAO (Food Agriculture Organization UN), WHO (World Health Organization), 2010). Environmental or geographic factors rather than genotypic ones seem to be meaningful in Pb bioaccumulation phenomena in rice grain (Zhang et al., 1996; Liu et al., 2003; Fangmin et al., 2006; Liu et al., 2013; Norton et al., 2014). Although the knowledge on the accumulation pathways for Pb in rice is rather approximate in comparison to As and Cd (Clemens and Ma, 2016), its variability in bioaccumulation seems to be smaller than that of As and Cd (Williams et al., 2009).

Oligoelements like Se, Ni, Cu, Mn and Mo are present in the paddy fields in total amounts ranging between thousands and few hundredths of mg kg^{-1}, as a function of the element and the pedologic origin of the soil (Spanu et al., 2018; Spanu et al., 2020; Ali et al., 2020; Wang et al., 2003; Cao and Hu, 2000; Cao et al., 2001; Chowdhury et al., 2017; Pan et al., 2016). The constant anaerobic conditions imposed by CF favor the presence of the reduced forms of each element in the paddy soil. In particular, the solubility of Ni, Cu, Mn and Mo in soils is controlled by precipitation of their insoluble compounds (mainly sulfides) (Ali et al., 2020; Alloway, 2013; Adriano, 2001), and the increase of soil alkalinity generally leads to further lower their concentrations in soil solution (Pan et al., 2016; Alloway, 2013; Adriano, 2001). On the other hand, SeO_4^{2-} and SeO_3^{2-} are the most common forms in which this nonmetal is present in the soil (Spanu et al., 2020; Ali et al., 2020), depending on both its redox potential and pH value.

If, until thirty years ago, Se was considered a contaminant like As, Cd, Pb, and Hg (McLaughlin et al., 1999), now it is rightly known as an essential trace element for biota and humans. Plant-derived foods represent its main dietary source and an insufficient intake of Se is associated with several human diseases (Hatfield et al., 2014); for these reasons, Se has become one of the principal targets of food biofortification strategies (Malagoli et al., 2015). Se bioaccumulation in rice grain is very sensitive to changes both in the irrigation method and in genotypes. In a very recent study, the adoption of SP irrigation was able to reduce by 90% the Se bioaccumulation in grains with respect to that measured for rice irrigated by CF, whereas a wide variability as a function of the genotype has been evidenced (Spanu et al., 2020).

Due to its wide use in many industrial applications, humans are always exposed to Ni by several different sources and pathways. Ingestion is the major one, but most of Ni is not absorbed, since it is efficiently removed by the kidneys and the gastrointestinal tract. On the other hand, Ni compounds are human carcinogens for inhalation (Brown et al., 1987; IARC, 2012), whereas Ni metal and its compounds are worldwide renowned contact allergen (Thyssen et al., 2007). In addition, highly sensitized people may also be interested by allergies caused by Ni contained in foods (American Academy of Dermatology, 2015). The concentration of Ni in rice grain has been seldom measured: its average amount is around 0.4 mg kg^{-1}, and its range is between 0.045 mg kg^{-1} and 3.460 mg kg^{-1} (Wang et al., 2014; Fu et al., 2008; Zhao et al., 2010; Rahman et al., 2014; Phuong et al., 1999).

Trace elements such as Cu, Mn, and Mo are involved in several cellular and molecular processes (Yruela, 2005; Campbell and Nable, 1988; Mendel, 2011; Cersosimo and Koller, 2006). A deficiency or an excess of these elements severely impairs plant growth and development. Since they have also a great technological importance in key technological sectors, the possibility of environmental contamination is realistic. Mn overexposure can cause a rare neurological and biphasic disorder called “manganism” (Li et al., 1994). While the acute Cu toxicity in humans can determine the generation of reactive oxygen species able to damage DNA (Roberts and Schilsky, 2008), chronic toxicity is observed only in patients bearing autosomal recessive mutations occurring in Cu transport proteins, leading hence to Wilson's disease (Roberts and Schilsky, 2008). Subjects with Alzheimer's disease can see worsening their symptomatology in presence of elevated levels of Cu (Brewer, 2012). Toxic effects by Mo strongly depend on the chemical state of the element. Animal studies suggest that chronic ingestion of more than 10 mg day^{-1} of Mo can damage the lungs, kidneys, and liver, causing diarrhoea, infertility, gout, low birth weight and growth retardation (Barceloux and Barceloux, 1999). In addition, high levels of Mo can interfere with the body's uptake of Cu, producing its deficiency (Suttle, 1974). Also the determination of the amounts of Mn, Cu and Mo in rice is not common: the concentration of these elements in 323 samples of rice grain produced worldwide spanned between 2 and 60 mg kg^{-1}, 0.5 and 13 mg kg^{-1}, 0.108 and 3.050 mg kg^{-1}, respectively (Zhao et al., 2010).

While the amount of trace elements has been measured in commercial and rural rice (Fu et al., 2008; Zhao et al., 2010; Frazzoli et al., 2006; Teklić et al., 2013; Qian et al., 2010; Jorhem et al., 2008a; Jorhem et al., 2008b; Jo and Todorov, 2019; Halder et al., 2020), at best of our knowledge no previous contribution has been addressed to the simultaneous measurement of the total amount of a representative group of toxic and potentially toxic elements contained in rice from many different genotypes produced using both conventional and intermittent irrigation methods. For these reasons, the principal aim of this study is to investigate changes in bioaccumulation of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in grains from a wide number of different rice genotypes cultivated in the same soil-water system, varying the irrigation method (i.e. CF, SP and SA). To do this in the most reliable way, an original and fully validated ICP-MS method aimed to measure the total amount of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in rice grain has also been developed.

2. Materials and methods

2.1. Site, soils, irrigation water and Rice genotypes

Rice cultivation was accomplished in 2012 at the University of Sassari's experimental farm “Santa Lucia”, Sardinia, Italy (39°59′ N, 8°40′ E; 15 m AMSL). The climate of the site is mediterranean, characterized by scarce precipitations (less than 150 mm in the cultivation period, i.e. May–September) and rather high mean temperatures (the average values in the last ten years were between 18.8 °C and 23.7 °C in the period May – September). The soil is a *Pantofluvic Eutric Fluvisol Loamic*, according to the World Reference Base for Soil Resources' classification (FAO, 2014). The areas devoted to CF or SA irrigation have been traditional paddy fields for the last 35 years, but also the soils used for cultivating rice with SP irrigation, contiguous to those used for CF and SA irrigation, were clayey with good water retention capacity. All experimental fields were similar in terms of pedological classification as well as hydrological and chemical properties. Irrigation water came exclusively from Lake Omodeo (40°08′10″N, 8°54′54″E), an artificial basin located in the center of Sardinia along the Tirso River. Twenty-six genotypes were cultivated: twenty of them were belonging to the Japonica subspecies (i.e. *Aleramo, Antares, Balilla, Brio, Carnaroli, Carnise, Cerere, CRV 04, CRV 108, CRV 114, CRV 390, Galileo, Gloria, Luxor, Musa, Opale, Orione, Ronaldo, Selenio* and *Virgo*) and the remaining six belonging to the Indica subspecies (i.e. *Apollo, Oceano, Salvo, Sprint, Thaibonnet* and *Urano*). With the only exceptions of the four CRV genotypes, the denominations of the remaining ones are the same reported in the Italian Register of Rice Varieties (Servizio Informativo Agrario Nazionale (SIAN), 2018).

2.2. Irrigation methods

2.2.1. Continuous flooding irrigation (CF)

This is the irrigation method used worldwide. The field was laser-leveled and surrounded by embankments to contain irrigation water. Plots were kept flooded with approximately 10 cm of water from seeding to 10–15 days before harvest (i.e. until when the most late-ripening cultivar had reached its full ripening). The redox potential of a CF soil was held constantly negative (usually at −200 mV vs. SCE), hence indicating the prevalence of reductive conditions. Each time the water level decreases to 2–3 cm, the addition of water restores the initial level.

2.2.2. Saturation irrigation (SA)

The soil was never flooded, but it was cyclically saturated whenever the top 5 cm were dry. The amount of irrigation water provided was equal to the soil's water storage capacity – i.e., the volume of water needed to fill its pores. The field must be leveled, but perimetral embankments were not required. To let the water spread homogeneously on the field, 10 cm-deep furrows were dug every 25–30 m. Water flowed in the furrows and saturated the plots. By using this irrigation method, evaporation and percolation losses were minimized. The field has been saturated at least once a week. In the time elapsed between two consecutive saturations, the redox potential of soil followed a roughly cyclical behavior, typically ranging between −100 and + 400 mV vs. SCE. Usually, the redox potential of the soil remained negative for the first few days after each saturation, then it assumed positive values that continuously increased until the next saturation.

2.2.3. Sprinkler irrigation (SP)

In this case the soil was always in oxidized conditions, because it was never flooded or saturated. Its redox potential was usually between 100 and 200 mV vs SCE. In order to avoid the bedding of rice, water was sprayed into the air by means of low- (or medium-) flow sprinkler heads, and letting it fall on the soil as rainfall. In each irrigation cycle, water was provided until soil reached its field capacity, and this was accomplished when only half the volume of the soil pores was occupied by water. The periodicity of the irrigation and the amount of water provided in each cycle was determined on the basis of the amounts of water evaporated by a Class A Evaporation pan (E_oA), corrected by a growing stage-specific constant k_c ranging from 0.4 to 1.2, as a function of the phenological phase of rice (Spanu et al., 2009).

Additional details on the three irrigation methods used have been provided elsewhere (Spanu et al., 2018; Spanu et al., 2020).

2.3. Experimental design, crop management and harvesting

The experimental design was a randomized block with 4 replications for each genotype. The surface of each sub-plot was 10 m^2. The preparation of the seed bed was performed by chisel plowing to a depth of 20 cm, followed by a secondary tillage with a field cultivator. Sowing was performed on dry soil using a seed drill. Seeds were placed at a depth of 3 cm with 14 cm of inter-row distance, with 500 viable seeds m^{-2}. Fertilization and herbicide treatments were accomplished according to what has been already described in previous contributions (Spanu et al., 2018; Spanu et al., 2020). Harvesting was performed between the last days of September and first days of October by means of a small plot combine harvester.

2.4. Instrumentation

Rice and soil samples were mineralized by acid/oxidant-assisted microwave irradiation using an Ethos Easy Lab Station microwave oven (Milestone, Sorisole, Italy). The quantitative determination of As, Cd, Pb, Se, Ni, Cu, Mn and Mo was performed using a NexION 300X ICP-MS spectrometer (Perkin Elmer, Milan, Italy), equipped with a nebulization system composed of a glass concentric nebulizer, a glass cyclonic spray chamber, a S10 autosampler and a KED collision cell. The ICP-MS spectrometer was controlled by a proprietary software (NexION software Version 1.0) running under the Windows 7 environment. The redox potentials of the soils were measured using a Thermo Orion model 210A electronic millivoltmeter, connected to a Pt electrode and a Saturated Calomel reference electrode, SCE (Amel Instruments, Milan, Italy).

2.5. Reagents

Aqueous solutions of HNO_3 (67%), of HCl (37%) and of H_2O_2 (30%) were all Normatom reagents from VWR (Milan, Italy), and were used in all the phases of the study. Standard aqueous solutions of Cd, Pb, Se, Ni, Cu and Mn (1000 mg dm^{-3} in HNO_3) and standard aqueous solutions of As and Mo (1000 mg dm^{-3} in HCl) were from Fluka, Milan, Italy. High-purity water (type I, resistance >18 MΩ) was produced using a MilliQplus System (Millipore, Vimodrone, Italy) and was used for all the analytical phases of the study. The ICP-MS Setup Solution (a 1% (*v/v, aq*) solution of HNO_3 containing 1 μg dm^{-3} each of Be, Ce, Fe, In, Li, Mg, Pb and U), the KED Setup Solution (a 1% (*v/v, aq*) solution of HNO_3 containing 10 μg dm^{-3} of Co and 1 μg dm^{-3} of Ce) and the internal standard solution (containing 10 μg dm^{-3} of Rh in a 1% (*v/v, aq*) solution of HNO_3 were all from Perkin Elmer. Certified rice flours by NIST SRM 1568a (National Institute of Standards and Technology, Gaithersburg, MA, USA), by IRMM 804 (European Commission Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium), by NCSZC (codes 73,008 and 11,007, both produced by the China National Analysis Centre, Beijing, China), were used in this study, whereas a certified soil SS-1 EnviroMAT contaminated soil was from SCP Science (Baie D'Urfé, QC, Canada).

2.6. Sampling and analytical methods

Irrigation waters were sampled according to the APAT- IRSA CNR methods (APAT-IRSA CNR Metodi Analitici per le Acque et al., 2003) and analyzed according to a literature method (Birke et al., 2010). Sampling of the soils was accomplished at the depth of 0–20 cm according to the official methods for the soil analysis of the Italian Republic (Gazzetta Ufficiale Serie Generale, 1999). Hydrological, physico-chemical and main chemical parameters were accomplished by literature methods (Spanu et al., 2004a; Birke et al., 2010; Gazzetta Ufficiale Serie Generale, 1999; Pansu and Gautheyrou, 2006; Spanu et al., 2004b). Analytical rice samples were obtained by quartering the paddy rice obtained from each sub-plot. The samples were then dried at 32 °C, mechanically husked and bleached. Original methods described in the Results section were used to measure the total concentration in rice grain and in soil samples of As, Cd, Cu, Mn, Mo, Ni, Pb and Se. Further details on the sampling technique and the analytical methods used are available in the Supplementary Material.

2.7. Statistical analysis and chemometrics

A two-tail *t*-test ($p = 95\%$) was used in the evaluation of bias existence. Principal Component Analysis (PCA) of data obtained on rice samples was performed using the CAT (Chemometric Agile Tool) software (Leardi et al., 2020).

3. Results and discussion

3.1. Assessment and optimization of the analytical procedure for elemental analysis in rice and soils

The procedures of mineralization of both soils and rice samples have been accomplished based on methods previously developed by this research group (Spanu et al., 2020). For soils, the use of HCl and HNO_3 as mineralizing reagents did not allowed to dissolve the soil silicates, hence the extractable fraction of the elements was measured. For rice grains, high amounts (1.4 g) of sample have been mineralized in order to concentrate the resulting solution mainly in the elements whose concentration is expected to be less abundant, i.e. for As, Cd, Pb and Se. Water (4 cm^3) was also added to the mixture to minimize the evolution of gases caused by the high amount of saccharides (up to 80% in weight) contained in rice. Furthermore, in the attempt to reduce the pressure in the vessel during the phase of microwave-assisted mineralization, a preliminary room temperature mineralization of the matrix has been performed overnight. 4 cm^3 of HNO_3 and 2 cm^3 of H_2O_2 were added to the vessel containing a weighted amount of a CRM rice flour NIST SRM 1568a, hence the microwave-assisted mineralization cycle was performed. Given the low recoveries of Cd observed, this last procedure was abandoned.

The success of the ICP-MS method depends in an utmost way by a careful selection of the ionic mass of the analytes. As a matter of fact, not always the best choice is represented by the most abundant isotope, and this is due to many possible ions generated in the Ar plasma, like those either by isobaric, by double charge, by refractory and molecular ions, all potentially able to generate a severe bias by overestimation (Becker, 2008). For As and for Mn the choice is obliged, since both elements are monoisotopic (^{75}As and ^{55}Mn). For the remaining six elements, the candidate isotope should couple a good abundance with the substantial absence of any severe interference. ^{111}Cd has been chosen as candidate isotope given the absence of isobaric and spectral interferences showed by all the most abundant Cd isotopes, whereas ^{63}Cu has been selected given the highest abundance (69.17%) among the relevant isotopes and also for the scarce probability of the formation of both the interfering molecular ions $^{31}P^{16}O_2^+$ and $^{47}Ti^{16}O^+$. The most abundant isotope of Mo (out of seven naturally present) is ^{98}Mo (abundance: 24.13%), isobarically interfered by $^{98}Ru^+$. Since the substantial absence of reliable molecular interferences and the existence of a simple correction equation useful to compensate the isobaric interference, $^{98}Mo^+$ has been chosen as quantification ion. Among all the isotopes of Ni, ^{60}Ni is the best choice, since it is the most abundant one and the only molecular interference is represented by the improbable $^{44}Ca^{16}O^+$ ion. Also ^{208}Pb is the most abundant isotope (52.40%) of this element. The fact that it is also free of any reliable polyatomic interference makes it ideal for any ICP-MS analysis. The reasons for the choice of ^{82}Se for the quantification of this element have been thoroughly discussed in a previous contribution (Spanu et al., 2020). Due to the well-known interferences by molecular ions (i.e. $^{40}Ar^{35}Cl^{+}$ and, in a minor extent, $^{59}Co^{16}O$ and $^{39}K^{36}Ar$ on the monoisotopic $^{75}As^{+}$, $^{81}Br^{1}H^{+}$ and $^{40}Ar_2H^{+}$ on $^{82}Se^{+}$), both the determinations of As and Se were performed using a kinetic energy discrimination (KED) of the analyte ion by the interfering molecular ion, accomplished using a flow of He able to promote the fragmentation of the molecular ion, removing hence any interference. The optimization of the He flow (typically few $cm^3\ min^{-1}$) is needed, because a low flow is unable to completely remove the interference, whereas high flow reduces also the signal by the analyte. Fig. 1S in the Supplementary Material reports the behavior of the counts for second (cps) of *m/z* 75 and m/z 82 signals from a solution containing both 10 μg dm^{-3} of As and Se at varying of the He flow. It is evident that an He flow of 3.5 $cm^3\ min^{-1}$ is able to contemporarily remove both the interferences. On the other hand, Table 1S in the Supplementary Material summarizes the elemental settings used for the method assessment.

The signals of the remaining elements (i.e. Cd, Cu, Mn, Mo, Ni and Pb) were acquired in normal mode. Since neither a significant difference (two-tail *t*-test, $p = 0.95$) has been found between the slopes of the regression lines obtained by external calibration and multiple standard additions, nor any meaningful bias was found by application of this method to the rice flour CRM NCSZC 73008, quantification in this matrix was accomplished for all analytes using the external calibration. The absence of bias also for Mn (i.e. the only monoisotopic element measured in normal mode) proved that the presence of several molecular ions potentially interfering in its determination is inexistent. On the other hand, quantification of the extractable amount of analytes in the soils has been accomplished by means of the multiple addition of standard solutions, owing to the presence of a matrix interference for some of them. Furthermore, a careful optimization of the nebulizer gas flow has allowed to minimize the oxide ions and double charge ions, reducing hence the interference in determination of Cd, Cu, Mn, Mo, Ni and Se. Details on the quality control of the analytical data are available in the Supplementary Material.

3.2. Validation

Validation of the analytical method aimed for the determination of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in rice and soil has been accomplished in terms of LoD, LoQ, linearity, precision and trueness. Validation results for rice and soil are shown in Tables 1 and 2, respectively.

The method for the determination of elements in rice was characterized by very low LoD values, ranging between 0.08 μg kg^{-1} (Mo) and 0.62 μg kg^{-1} (Se), measured according to Currie (Currie, 1999). The LoD values of toxic elements were always below 0.2 μg kg^{-1}. Whilst a concentration range between 1 and 200 μg kg^{-1} was found to be suitable for analyzing toxic elements and Se without any further dilution another interval, slightly shifted towards higher concentrations (between 10 and 500 μg kg^{-1}) has been used for the more abundant elements (i.e. Cu, Mn, Mo and Ni). In these intervals of concentration,

Table 1
Validation parameters for the ICP-MS determination of the total amount of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in rice grains.

Element	LoD[a] (μg kg^{-1})	LoQ (μg kg^{-1})	Linearity[b] $Y = (a \pm s_a^{f})X + (b \pm s_b^{f})$; R^2	Repeatability[c] C, certificated concentration		Intermediate precision[d] C, certificated concentration		Trueness[e] C, certificated concentration	
				CRM (C, mg kg^{-1})	*RSD*	*CRM (C, mg kg^{-1})*	*RSD*	*C, mg kg^{-1}*	*Recovery (%)*
As	0.17	0.55	$a = 777$; $s_a = 7$	NCSZC 11007 (0.11)	1.7	NCSZC 11007 (0.11)	3.7	0.11 ± 0.02	105 ± 4
			$b = 140$; $s_b = 200$	NCSZC 73008 (0.10)	13	NCSZC 73008 (0.10)	15	0.102 ± 0.008	105 ± 6
			$R^2 = 0.9992$	NIST 1568a (0.29)	3.4	NIST 1568a (0.29)	2.6	0.29 ± 0.03	103 ± 4
				IRMM 804 (0.049)	1.2	IRMM 804 (0.049)	2.3	0.049 ± 0.004	99 ± 3
Cd	0.11	0.35	$a = 8300$; $s_a = 600$	NCSZC 11007 (1.28)	4.5	NCSZC 11007 (1.28)	3.2	1.28 ± 0.03	105 ± 7
			$b = 2000$; $s_b = 2000$	NCSZC 73008 (0.09)	11	NCSZC 73008 (0.09)	17	0.087 ± 0.005	94 ± 8
			$R^2 = 0.9993$	NIST 1568a (0.36[g])	5.2	NIST 1568a (0.36[g])	8.1	0.022 ± 0.002	101 ± 4
				IRMM 804 (1.61)	0.8	IRMM 804 (1.61)	1.7	1.61 ± 0.07	95 ± 5
Cu	0.29	0.96	$a = 39{,}400$; $s_a = 400$	NCSZC 11007 (3.1[g])	5.6	NCSZC 11007 (3.1[g])	1.4	h	
			$b = 28{,}000$; $s_b = 30{,}000$	NCSZC 73008 (4.9)	6.5	NCSZC 73008 (4.9)	8.1	4.9 ± 0.3	90 ± 7
			$R^2 = 0.9998$	NIST 1568a (2.4)	4.5	NIST 1568a (2.4)	2.8	2.4 ± 0.3	88 ± 9
				IRMM 804 (2.74)	7.7	IRMM 804 (2.74)	3.6	2.74 ± 0.2	92 ± 6
Mn	0.31	1.0	$a = 118{,}000$; $s_a = 3000$	NCSZC 11007 (11.6[g])	5.0	NCSZC 11007 (11.6[g])	1.7	h	
			$b = 70{,}000$; $s_b = 80{,}000$	NCSZC 73008 (17)	5.2	NCSZC 73008 (17)	6.1	17 ± 1	89 ± 8
			$R^2 = 0.9963$	NIST 1568a (20)	3.9	NIST 1568a (20)	7.6	20.0 ± 1.6	88 ± 9
				IRMM 804 (34.2)	3.3	IRMM 804 (34.2)	3.4	34.2 ± 2.3	97 ± 4
Mo	0.08	0.27	$a = 24{,}400$; $s_a = 300$	NCSZC 11007 (0.46[g])	11	NCSZC 11007 (0.46[g])	3.2	h	
			$b = 30{,}000$; $s_b = 30{,}000$	NCSZC 73008 (0.53)	7.2	NCSZC 73008 (0.53)	8.4	0.530 ± 0.050	94 ± 8
			$R^2 = 0.9998$	NIST 1568a (1.46)	3.1	NIST 1568a (1.46)	7.6	1.460 ± 0.08	86 ± 8
				IRMM 804 (0.3363[g])	0.2	IRMM 804 (0.3363[g])	3.4	h	
Ni	0.25	0.83	$a = 18{,}800$; $s_a = 150$	NCSZC 11007 (0.6[g])	**34**	NCSZC 11007 (0.6[f,g])	2.3	h	
			$b = 2500$; $s_b = 3000$	NCSZC 73008 (0.27)	17	NCSZC 73008 (0.27)	12	0.27 ± 0.02	93 ± 6
			$R^2 = 1.0000$	NIST 1568a (0.2[g])	**50**	NIST 1568a (0.2[g])	**45**	h	
				IRMM 804 (0.184[g])	4.6	IRMM 804 (0.184[g])	23	h	
Pb	0.09	0.29	$a = 58{,}000$; $s_a = 3000$	NCSZC 11007 (0.10)	7.1	NCSZC 11007 (0.10)	6.6	0.10 ± 0.01	87 ± 6
			$b = 6000$; $s_b = 8000$	NCSZC 73008 (0.08)	18	NCSZC 73008 (0.08)	16	0.08 ± 0.03	85 ± 7
			$R^2 = 0.9998$	NIST 1568a (0.0133[g])	4.3	NIST 1568a (0.0133[g])	**36**	h	
				IRMM 804 (0.42)	2.5	IRMM 804 (0.42)	6.7	0.42 ± 0.07	96 ± 8
Se	0.62	2.0	$a = 52.0$; $s_a = 0.3$	NCSZC 11007 (0.17[g])	12	NCSZC 11007 (0.17[g])	14	h	
			$b = 0.3$; $s_b = 0.8$	NCSZC 73008 (0.06)	3.5	NCSZC 73008 (0.06)	7.0	0.061 ± 0.015	107 ± 8
			$R^2 = 0.9992$	NIST 1568a (0.38)	2.4	NIST 1568a (0.38)	8.2	0.38 ± 0.04	99 ± 2
				IRMM 804 (0.058[g])	11	IRMM 804 (0.058[g])	20	h	

Precision values in **bold** are not acceptable in terms of Horwitz's theory (Horwitz, 1982).
[a] The LoD value is measured according to Currie (1999).
[b] The linearity range is between 1 and 200 μg kg^{-1} for As, Cd, Pb and Se, and between 10 and 500 μg kg^{-1} for Cu, Mn, Mo and Ni.
[c] Evaluated by analyzing four different CRM five times within the same analytical session.
[d] Evaluated by analyzing four different CRM fifteen times in five different analytical sessions within one month
[e] Evaluated by analyzing three aliquots of CRM rice flour within the same analytical session.
[f] Standard deviation.
[g] The data reported is not the certified concentration, but the amount measured by this method.
[h] Data not certificated.

Table 2
Intermediate precision and trueness for the ICP-MS determination of the total amount of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in soils.

Element	Enviromat Contaminated Soil SS-1 *(C ± SD[c], mg kg^{-1})*	Intermediate Precision[a] *RSD*	Trueness[b] *Recovery ± SD[c] (%)*
As	20.7 ± 1.0	5.3	95 ± 6
Cd	3.2 ± 0.2	7.5	94 ± 4
Cu	4033 ± 10	3.3	97 ± 6
Mn	737 ± 19	4.8	94 ± 7
Mo	6.8 ± 0.3	8.9	90 ± 4
Ni	59.2 ± 1.3	6.0	92 ± 8
Pb	764 ± 15	4.1	99 ± 5
Se	0.78 ± 0.14	12	87 ± 4

[a] Evaluated by analyzing the soil CRM ten times in five different analytical sessions within one month.
[b] Evaluated by analyzing three aliquots of CRM rice flour within the same analytical session.
[c] Standard deviation.

very good values of R^2, almost always higher than 0.9992, have been found. Furthermore, a random dispersion of residuals of the regression line around zero supports linearity for the methods proposed. Precision and trueness were always measured on CRMs of the matrices under exam. Precision, evaluated in terms of repeatability and intermediate precision, has been found to be acceptable according to Horwitz's theory (Horwitz, 1982), whereas trueness was evaluated analyzing CRMs' rice flour and soils, provided quantitative recoveries (criteria: two tails *t*-test, $p = 0.95$).

3.3. Analytical determinations on soils and irrigation waters

Table 3 reports the hydrological, the physico-chemical, and the chemical characterization of the soils used for the experimentation, as well as the average amounts of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in the irrigation water.

The surface horizons of experimental soils have a sandy-clay texture, a subalkaline pH, a very low amounts of carbonates and bicarbonates, an amount of organic carbon and total nitrogen below 1.5% and 0.1%, respectively, whereas the amounts of assimilable phosphorus and exchangeable potassium were sufficient for the requirements of the cultivation of rice. Whereas the amounts of almost all elements in all experimental soils were within the typical range observed worldwide for uncontaminated soils (Alloway, 2013; Adriano, 2001), the amount of Mo was slightly below the lower limit of this range. Furthermore, relevant differences among the concentrations of the analytes within the three soils considered in this experimentation were observed. It is interesting to observe that, in soils used for SP irrigation, the amount of Mn and Ni is significantly higher than those measured in both CF and SA soils. This is likely since the latter soils have been used for CF consecutively for tens of years, and the constant flooding might have caused the slow leaching of the quite soluble reduced forms for both elements. In addition, the lack of any deep plowing, seldom practiced in paddy fields, has prevented the soil to its regeneration from elements leached in the years. Finally, the concentrations of all elements measured in the irrigation waters ranged by around one tenth of μg kg^{-1} and few tens of μg kg^{-1}, hence substantiating no situation of environmental alert.

3.4. Elemental concentrations of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in rice grain

The concentration ranges and the average amounts of each element considered measured in 26 rice genotypes when varying the irrigation method are reported in Table 4, whereas the tables reporting all analytical data for each rice genotype and irrigation method (Tables 2S–4S) are available in the Supplementary Material.

Data reported substantiated the very large variability of the total amount of the elements considered in rice grain. Among toxic elements, As exhibited the widest variability, since its concentrations spanned from 1 μg kg^{-1} to 200 μg kg^{-1}, with an average amount, calculated on all genotypes and all the irrigation methods, of 70.7 μg kg^{-1}. The amounts of Cd were between 1 μg kg^{-1} and 80 μg kg^{-1}, with a mean

Table 3
Typical hydrological, physico-chemical and chemical parameters characterizing the three soils used for the experimentation, and the average amounts of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in the irrigation waters. CF, continuous flooding irrigation, SA, saturation irrigation, SP, sprinkler irrigation.

Parameter	Soils CF	Soils SA	Soils SP	Irrigation water
pH	7.8	7.4	7.6	7.7
Redox potential[a] (mV vs. SCE)	−215	−120[b]; 370[c]	110[d]	e
Carbonates (% as $CaCO_3$)	<0.01	<0.01	0.01	0.008[f]
Total nitrogen (%)	0.05	0.02	0.09	e
Organic carbon (%)	1.2	1.4	1.3	e
Assimilable phosphorous (mg kg^{-1} as P_2O_5)	126	132	89	<0.5
Exchangeable potassium (mg kg^{-1} as K_2O)	205	215	210	2.3
Field capacity (%, v/v)	e	e	37.1	e
Permanent wilting point (%, v/v)	e	e	19.6	e
As (μg kg^{-1})	2000 ± 200[g]	2600 ± 200[g]	1990 ± 70[g]	0.09 ± 0.02[h]
Cd (μg kg^{-1})	180 ± 6[g]	132 ± 8[g]	100 ± 4[g]	0.092 ± 0.009[h]
Cu (μg kg^{-1})	15,000 ± 1000[g]	12,000 ± 1000[g]	15,600 ± 500[g]	1.6 ± 0.1[h]
Mn (μg kg^{-1})	37,000 ± 2000[g]	43,000 ± 3000[g]	105,000 ± 2000[g]	35 ± 3[h]
Mo (μg kg^{-1})	310 ± 20[g]	910 ± 70[g]	230 ± 20[g]	0.62 ± 0.07[h]
Ni (μg kg^{-1})	13,000 ± 1000[g]	20,000 ± 2000[g]	33,000 ± 1000[g]	1.10 ± 0.04[h]
Pb (μg kg^{-1})	7300 ± 300 [g]	7100 ± 500[g]	8500 ± 300[g]	2.03 ± 0.05[h]
Se (μg kg^{-1})	1700 ± 100[g]	1300 ± 70[g]	1130 ± 90[g]	1.19 ± 0.07[h]

If otherwise not reported, all parameters were evaluated according to ref. Gazzetta Ufficiale Serie Generale (1999).
[a] Redox potential was measured according to ref. Pansu and Gautheyrou (2006);
[b] Measured five hours after saturation
[c] Measured just before saturation.
[d] Measured halfway through each sprinkler cycle.
[e] Parameter not measured.
[f] Data relative to the concentration of HCO_3^- ion.
[g] Measured with the method developed in this work.
[h] Measured according to ref. Birke et al. (2010).

Table 4
Ranges and average concentrations of As, Cd, Cu, Mn, Mo, Ni, Pb and Se in rice kernels as a function of the irrigation method.

	CF (μg kg^{-1})		SA (μg kg^{-1})		SP (μg kg^{-1})	
	Average	Range	Average	Range	Average	Range
As	140	90–200	70	40–110	2.2	1–3
Cd	14	4.9–31	40	16–80	7.1	1.1–14
Cu	3200	1900–3800	3400	2900–4000	3100	2800–3800
Mn	7800	5300–11,000	11,500	7800–14,800	800	200–1600
Mo	880	500–1600	650	440–940	360	280–580
Ni	90	30–180	270	170–400	670	400–1200
Pb	32	7–140	19	10–40	16	8–30
Se	90	65–140	110	75–145	8.7	4.8–14

concentration of 20.4 μg kg^{-1}. Finally, Pb concentration in kernels ranged between 7 μg kg^{-1} and 140 μg kg^{-1}, with an average amount of 22.3 μg kg^{-1}. On the other hand, the highest variability on the amount of oligoelements considered (roughly around two magnitude orders) were observed for Mn (range between 200 and 14,800 μg kg^{-1}, average concentration of 6700 μg kg^{-1}) and for Ni (range between 30 and 1200 μg kg^{-1}, average concentration of 343 μg kg^{-1}), while the lowest variability was shown by Cu, whose amount spanned between 1900 and 4000 μg kg^{-1}, with an average concentration of 3223 μg kg^{-1}.

3.5. Chemometric interpretation of data

A PCA analysis has been performed on a data set made by 78 rows (the 26 genotypes, for each of the three irrigation methods) and eight columns (the eight elements).

After autoscaling, the first two components account for 51.6% and 21.1% of the total variance, respectively. The three irrigation methods are clearly differentiated on the score plot on the plane PC1-PC2 (Fig. 1a), with PC1 differentiating rice samples irrigated by SP by those irrigated by CF and SA, whereas the second component differentiates between samples irrigated by CF and SA. From the loading plot (Fig. 1b) the inverse correlation in the bioconcentration of elements in rice between Ni and most of the elements can be detected. It is therefore clear that with the SP irrigation lower amounts of Mn, Se, As and Mo, together with higher amounts of Ni, are obtained. On the other side, comparing SA and CF, the former gives higher amounts of Cd, Cu, Mn, Ni and Se. It can also be seen that with the SP irrigation the effect of the genotype is smaller than with the other methods. Fig. 2 shows zoomed images of the score plot reported in Fig. 1a.

It is not possible to detect a common distribution of the genotypes in the clouds of the three irrigation methods, except for *Balilla* and *Cerere* ones (in the score plot, Ba e Ce, respectively) always having the highest score on PC2, this meaning the lowest amounts for Cu, Pb and Mo. This suggests that the effect of each irrigation methods is different for each genotype. Finally, as reported in Fig. 2S, available in the Supplementary Material, the scores of Indica genotypes irrigated by CF or SP were closely grouped, suggesting in this case a their rather homogeneous behavior with respect to the bioaccumulation of the analytes in rice grains.

3.6. Influence of the irrigation method

3.6.1. Toxic elements

As preliminarily showed by the score plot of PCA (Fig. 1a), the data reported in Table 4 give account for a marked effect played by the three irrigation methods on the bioaccumulation of both toxic elements and oligoelements. Regarding toxic elements, it is evident that the adoption of intermittent methods of irrigation is effective in reducing the total amount of As, Cd and Pb in rice grain. In this context, SP is largely more effective than SA (90 vs 30% of reduction in the total amount of toxic elements found in rice irrigated by CF, respectively). In detail, the adoption of SP instead of CF allows the contemporary minimization of the average concentrations of all toxic elements in rice grain (−50% for Cd, −75% for Pb and, mainly, −98.4% for As). These data are in excellent agreement with those elsewhere obtained by the same research group for As on 37 genotypes irrigated by either CF and SP (Spanu et al., 2012), whereas the data measured for Cd are markedly better than those previously obtained by the same research group (i.e. 13% and 28% in the two years of the experiment, respectively (Spanu et al., 2018)). The meaningful reduction of the Cd bioaccumulation always observed in rice grain when SP irrigation was used cannot be ascribed to effects related to soil alkalization (Honma et al., 2016; Reddy and Patrick Jr., 1977; Kirkham, 2006) or formation of insoluble species like $CdCO_3$ (Wu et al., 2016; Li et al., 2020), and this is supported by the substantial constancy of the typical pH values (7.6 for SP soil, 7.8 and 7.4 for CF and SA soils, respectively) and the very low amounts of limestone (never higher than 0.01%) measured for all the soils here considered. Finally, an average reduction of 50% on the Pb bioaccumulation in rice grains irrigated by SP was observed in comparison to the amounts measured in the same genotypes irrigated by CF. As for the effect of SA irrigation, this method is able to reduce significantly, in comparison to CF, the amount of As (−50%) and Pb (−40%) but it causes, as previously

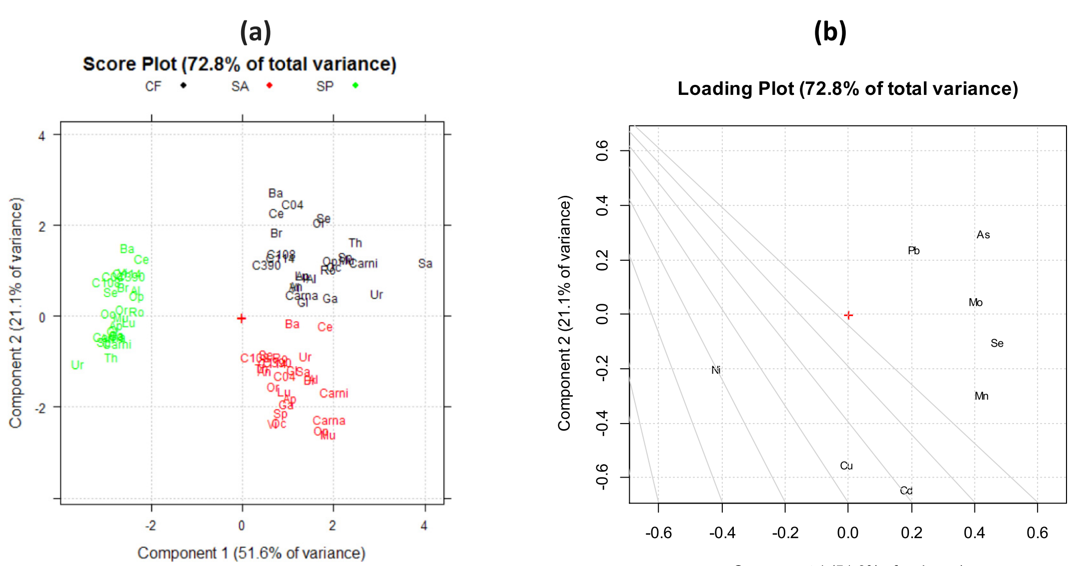

Fig. 1. Score plot (Fig. 1a) and loadings plot (Fig. 1b) of the concentrations of As, Pb, Cd, Cu, Mn, Mo, Ni and Se measured in rice grain of different 26 genotypes irrigated by CF, SA and SP.

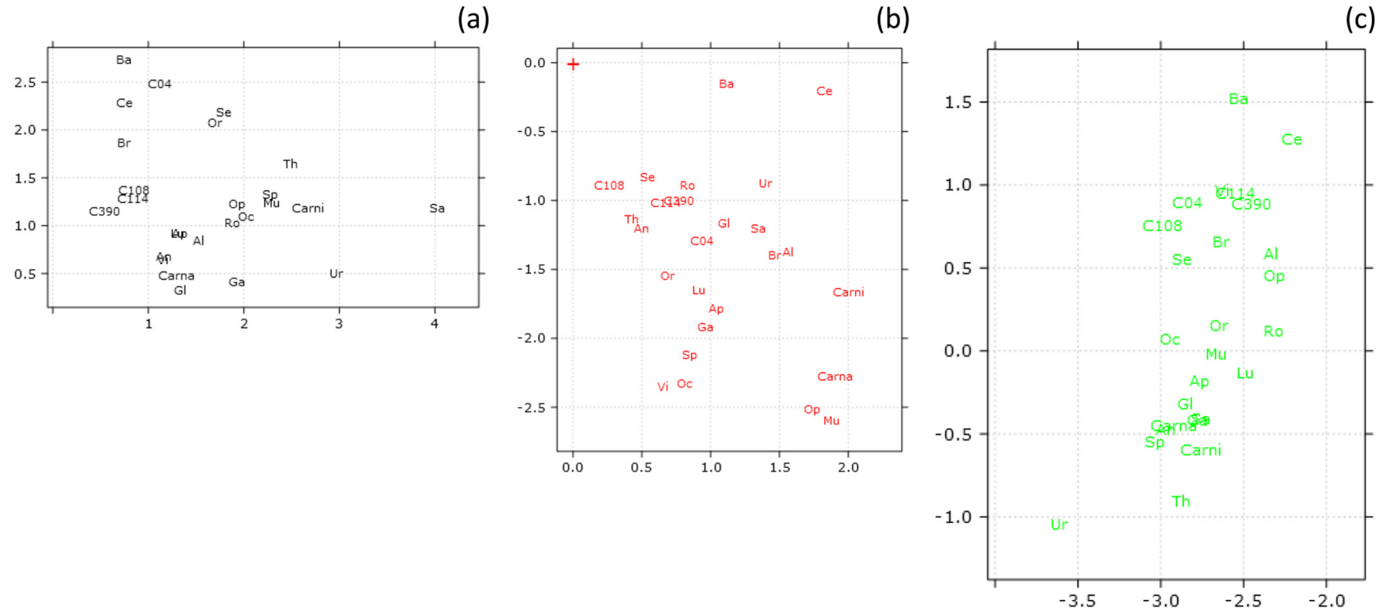

Fig. 2. Magnified details of the PC1-PC2 score plot relative to 26 rice genotypes: a), irrigated by CF; b) irrigated by SA; c) irrigated by SP.

observed (Spanu et al., 2018), a very meaningful increase of the Cd concentration (+285% vs that measured in CF rice).

Although studies of the effects on the bioaccumulation of Pb in kernels at varying the nature of the irrigation methods are unprecedented for this research group, a comparison of data obtained in this experimentation can be attempted with the data from the few contributions of literature on this topic (Liao et al., 2013; Ashraf et al., 2018; da Silva et al., 2020). In particular, data here reported are in quite good agreement with those reported by Ashraf et al. (Ashraf et al., 2018), who observed, on two rice genotypes, a meaningful reduction (between 37% and 52%) of the Pb amount in rice grain when CF irrigation was replaced with an irrigation method similar to SA (i.e., alternate wetting and drying, AWD, irrigation). Also Liao et al. (Liao et al., 2013) measured, in a two-years field experiment, a slight reduction (−13%) of the Pb concentration in rice spikes passing from CF to a Non-Flooding controlled Irrigation (NFI). On the other hand, da Silva et al. (da Silva et al., 2020), in a very recent three years-study conducted on open field with three rice genotypes and five irrigation methods (i.e. CF and four intermittent methods at increasing water tensions, all not too far from SA conditions) gave account, when the soil water tension was increased, for an increase of the Pb concentration on two rice genotypes. On the contrary, in the same conditions, a decrease of the Pb amount on the third genotype was also observed.

3.6.2. Oligoelements

Also the amount of oligoelements in rice grain was deeply influenced by changes in the nature of the irrigation methods. With the only exception of Ni, the adoption of SP irrigation in place of CF causes a reduction in the elemental bioaccumulation on rice grain. Hence, while the average amount of Cu measured in SP rice is reduced by only 3% in comparison to those measured in CF rice, the reduction was 59% for Mo, and 90% for both Mn and Se. On the other hand, the average concentration of Ni in rice grain irrigated by SP increased by 644% with respect to the amounts measured in rice irrigated by CF. The replacement of CF with SA increased the amount of Cu, Mn, Ni and Se in rice grain by 6% (Cu), 47% (Mn), 200% (Ni) and 22% (Se), whereas only for Mo a reduction of 26% was measured.

Even though for Se it is possible to make a comparison between the behavior here observed and that reported in previous years of experimentation (Spanu et al., 2020), this approach is not possible for Cu, Mn, Ni and Mo, hence data here reported for these elements have been compared with the closest literature studies (Liao et al., 2013; da Silva et al., 2020; Xu et al., 2019; Orasen et al., 2019). While an excellent agreement regarding the reduction of the Se bioaccumulation in rice grain between literature data and those reported in this study when passing from CF to SP has been observed, the increase of the amount of Se in kernels measured in this year replacing CF with SA is intermediate between that measured in the two years of the previous study (0% and 56%, respectively) (Spanu et al., 2020).

Among all elements considered in this study, Ni is the only one whose amount in rice grain was continuously increasing passing from CF to intermittent irrigation methods. The average amounts of Ni in rice irrigated with the three methods are very different, raising from the 90 $\mu g\ kg^{-1}$ for CF to the 670 $\mu g\ kg^{-1}$ for SP. Increases in the Ni bioaccumulation in kernels replacing CF with intermittent irrigations were found for all genotypes considered in this study. Data here reported are in good agreement with those reported by da Silva et al., (da Silva et al., 2020), who observed an increase of ca. 130% in the Ni amounts when decreasing the water tension from 0 kPa (i.e. in SA conditions) to −25 kPa (i.e. a condition roughly midway to SA and SP, being the latter condition correlated to a soil water tension typically between −35 and − 38 kPa (Parfitt et al., 2017)). Also Orasen et al. (Orasen et al., 2019) evidenced, on three different rice genotypes cultivated in two consecutive years, an average increase of the Ni concentration in rice grain between +300 and + 700% passing from CF irrigation to alternate wetting and drying irrigation.

The trend of Mn concentration in rice grain at varying the nature of the irrigation method somewhat resembles that of Cd, but with some meaningful differences. As a matter of fact, the change of the CF with the SA irrigation caused an increase of ca. 50% in the average Mn concentration in rice grain, whereas the replacement of CF with the SP irrigation caused an average drop of 90% in the concentration of Mn in kernels. The latter trend was basically confirmed by the results achieved by Xu et al. (Xu et al., 2019) and by Orasen et al. (Orasen et al., 2019), since both these groups observed a reduction between 25% and 50% of the Mn amount in rice grains when irrigations basically similar to CF (well-watered irrigations) were replaced with intermittent methods of irrigation quite similar to SP (like alternate wetting and moderate soil drying irrigation (Xu et al., 2019) or alternate wetting and drying irrigation (Orasen et al., 2019)). On the contrary, the results obtained by da Silva et al., (da Silva et al., 2020) showed, for the three rice genotypes considered, increases between 300% and ca. 40% passing from SA to

Fig. 3. Variability along the years of the ratio between the elemental concentrations measured in rice grain irrigated by intermittent methods (SA or SP) and CF ($R_{INT/CF}$ ratio, where INT = SA or SP) as a function of the rice genotype. Fig. 3a: Cd, three years, $R_{SA/CF}$ ratio, 26 rice genotypes; Fig. 3b: Cd, three years, $R_{SP/CF}$ ratio, 26 rice genotypes; Fig. 3c: As, two years, $R_{SP/CF}$ ratio, 9 rice genotypes; Fig. 3d: Se, three years, $R_{SA/CF}$ ratio, 26 rice genotypes; Fig. 3e: Se, three years, $R_{SP/CF}$ ratio, 26 rice genotypes. Fig. 3a and b: *Apollo, Oceano, Salvo, Sprint, Thaibonnet* and *Urano* rice genotypes belong to the Indica subspecies, whereas the remaining genotypes belong to the Japonica subspecies. Data relative to years 1 and 2 were calculated from ref. (Spanu et al., 2018), whereas those relative to year 3 were from this study. Fig. 3c: *Salvo* rice genotype belongs to the Indica subspecies, whereas the remaining genotypes belong to the Japonica subspecies. Data relative to year 1 were calculated from Spanu et al. (2012), whereas those relative to year 2 were from this study. Fig. 3d and e: *Apollo, Oceano, Salvo, Sprint, Thaibonnet* and *Urano* rice genotypes belong to the Indica subspecies, whereas the remaining genotypes belong to the Japonica subspecies. Data relative to years 1 and 2 were calculated from Spanu et al. (2020), whereas those relative to year 3 were from this study.

irrigation conditions somehow intermediate between SA and SP and characterized by soil water tensions roughly around −20 KPa.

Like As and Pb, also the concentration of Mo in rice grain decreases passing from CF to intermittent irrigations. In particular, the amount of Mo decreased by 26% when the CF was replaced by SA, and by 59% when SP was used in place of CF. This behavior has been also observed by Xu et al. (2019), that measured a significant reduction of the Mo amount (between 15% and 40% over two years) when the well-watered irrigation was replaced with the alternate wetting and moderate soil drying irrigation.

Among all elements considered in this study, Cu is the least sensitive to changes in the irrigation method. The average amount of 26 different genotypes of rice changes only a few percent points at varying of the irrigation method, and also the concentration ranges measured for each irrigation method are the narrowest among all elements studied and strongly overlapped among CF, SA and SP. These data are quite consistent with those found by Liao et al. (2013), who observed increases of Cu concentration ranging between 4 and 15% in a NJ 46 Japonica rice genotype passing from CF to NFI over two consecutive years. Much more pronounced increases (23%–80%) were observed by Orasen et al. (2019) in the cultivation of three rice genotypes for two years using CF and alternate wetting and drying irrigation methods. Conversely, Xu et al. (2019) observed reductions up to 60% of the amount of Cu in two genotypes of rice grain passing by well-watered irrigation to alternate wetting and moderate soil drying irrigation, i.e. a method which has common characteristics with SP. Since the variability observed among 26 genotypes in the ratio between the Cu concentrations measured for samples irrigated with SP and CF ranged between 73% and 135%, it is possible that other rice genotypes from those here considered can further minimize the bioaccumulation of Cu in rice grain. Also for Cu, the study of da Silva et al. (2020) led to results opposite to those reported in this study. A continuous increase of Cu concentration was measured at the ripening stage for all rice genotypes considered when the soil water potential was increased from values typical to saturation (0 kPa) to values halfway towards SP (i.e. between −17 kPa and − 25 kPa). Surprisingly, no significant variation was measured in the vegetative and reproductive phases.

3.6.3. Variability of Cd, As and Se bioaccumulation in rice grains among the agricultural years

As this research group has studied in previous agricultural years (Spanu et al., 2018; Spanu et al., 2012; Spanu et al., 2020) the dependence of the elemental bioaccumulation in rice grain of toxic elements like As and Cd, as well as of oligoelements like Se, as a function of either the irrigation methods and the rice genotypes, the variability along years for these elements is reported in Fig. 3 in terms of the $R_{INT/CF}$ ratio, i.e. the ratio between the elemental concentration measured in rice irrigated by an intermittent (INT = SA or SP) method and the same amount measured in rice irrigated by CF. Fig. 3a reports the trend for Cd of the $R_{SA/CF}$ ratio, whereas Fig. 3b reports the Cd trend for the $R_{SP/CF}$ ratio.

Fig. 3a reveals an extreme variability of the $R_{SA/CF}$ ratio within the three agricultural years considered. With the only exception of the CRV 390 rice genotype in the year 3 (data measured in this study), this ratio is always higher than 1, supporting a very large increase of Cd bioaccumulation passing from CF to SA irrigation. A $R_{SA/CF}$ ratio even higher than 30 was measured for the *Opale* genotype in year 2 (Spanu et al., 2018). A large variability along years, but with much less amounts of the relevant $R_{SP/CF}$ ratio, has also characterized Fig. 3b. Even if, in half of the cases, it is possible to observe $R_{SP/CF}$ ratios >1, the samples where the $R_{SP/CF}$ ratio become very high (e.g., higher than 2) are quite rare, and only in one case (always *Opale* genotype in year 2 (Spanu et al., 2018)) this ratio approaches to 5. Furthermore, the fact that eight rice genotypes have been able, in all three years, to constantly keep the $R_{SP/CF}$ ratio below 1, supports the overall capability of SP to reduce the bioaccumulation of Cd in rice grain also in comparison to the amounts obtained in samples irrigated by CF.

In a close analogy to Fig. 3a and b, Fig. 3c reports the behavior for As of the $R_{SP/CF}$ ratios for the common nine genotypes considered in the two years of experimentation for this element (year 1, data from (Spanu et al., 2012), year 2, data measured in this study). Contrarily to what found for Cd, a very high constancy of the $R_{SP/CF}$ ratio for As has been observed, that it is always between the narrow range of 0.010 and 0.023. Furthermore, it is noteworthy to observe that the amount of As measured in SP rice, always within 1 and 5 $\mu g\ kg^{-1}$, is not so different of those normally found in the unpolluted natural waters, and this supports hence an almost total absence of As bioaccumulation in rice grain in conditions of SP irrigation.

Also for Se it is possible to make a direct comparison of data obtained with those from a study recently published by this research group (Spanu et al., 2020). Fig. 3d and e report the behaviors of both the $R_{SA/CF}$ and $R_{SP/CF}$ ratios, respectively, for each genotype along the three years of experimentation. In them, data relative to years 1 and 2 were obtained by ref. (Spanu et al., 2020), whereas those relative to year 3 were measured in this study. Fig. 3d indisputably provides evidence of an increase of the amount of Se in rice grain passing from CF to SA irrigation. Although the amounts of the $R_{SA/CF}$ ratio never assume in this case the very high values observed for Cd, it is possible to note that there is no genotype which exhibits, for all the three years of observation, $R_{SA/CF}$ ratios always below 1. Interestingly, all $R_{SA/CF}$ ratios for year 2 are higher than 1, and this is likely due to the quite low amounts of Se measured for all rice genotypes irrigated with SP. On the other hand, Fig. 3e explains the strong tendency of SP to reduce also Se amounts in rice grain compared to CF. For almost all genotypes, with the only exceptions of those *Apollo, Opale, Ronaldo* and *Selenio* in year 2, the $R_{SP/CF}$ ratios were below 0.2, even if they never reached the least values previously observed for As. A similar behavior was observed also by Xu et al. (Becker, 2008), which accounted for a very significant reduction of the Se in rice grain passing from well-watered irrigation to an alternate wetting and moderate soil drying irrigation: as previously remarked, both irrigation methods seem to be not too far from CF and SP irrigation methods used in this study, respectively.

3.7. Influence of rice genotype and subspecies

Also the nature of the rice genotype plays a key role in the bioaccumulation phenomena of both toxic and trace elements in kernels. In particular, it has been demonstrated that the nature of the genotype and its interaction with the environmental variables influence up to 30% of the total amount of As in rice grain (Ahmed et al., 2010). In order to make the discussion of this effect easier, a normalization of the data reported in Tables 2S–4S, and summarized in Table 4, was performed. More precisely, the amounts of element found in each rice genotype were normalized with respect to the average amount measured for each irrigation method. Hence, the ranges of normalized bioaccumulation ratios (NBRs), calculated for each possible couple element-genotype and for each irrigation method used, were expressed as the ratio between the concentration C of the element X in the rice genotype Y and the average concentration $\overline{C}$ calculated for the same element on all genotypes considered, all irrigated with one of the three methods considered. Hence, NBR_{XY} (CF, SA or SP) = $C_{XY}/\overline{C}_X$ (CF, SA or SP). Table 5 reports the ranges of NBRxs for each element studied, for the sum of all the possible couples of toxic elements, for the sum of all toxic elements, and - finally - for the sum of all elements kept into exam in this study, whereas Tables 5S–7S reports all the NBRx values for each irrigation method considered. In these tables, data relative to Cd and Se are reported for a period of three years.

For all the irrigation methods, the *Virgo* genotype is the worst bioaccumulator of As (NBR_{As} ranging between 0.54 for SP and 0.64 for CF), whereas different are the genotypes best bioaccumulators of As at varying of the irrigation method (i.e. *Carnise* in CF; *Musa* in SA and *Opale* in SP, with NBR_{As} ratios of 1.42, 1.57 and 1.36, respectively). At least for CF irrigation, these results are in good agreement with those from literature. As an example, the NBR_{As} range calculated over several rice genotypes cultivated in polluted sites of Bangladesh (Norton et al., 2009), of India and China (Ishikawa et al., 2016) ranged between 0.17 and 2.52 and between 0.64 and 1.80, respectively. Relevant differences in bioaccumulation of As were found between red bran and brown bran rice genotypes (Norton et al., 2009). Although *Virgo* genotype seems able to intrinsically reduce the bioaccumulation of As in all the irrigation methods, this capability tends to be increased passing from CF to SA and – finally – SP methods. Furthermore, this genotype minimizes contemporary the concentration of As, Cd and Pb only in SP irrigation, because in SA and – mainly – in CF irrigation, the *Virgo* genotype is a good bioaccumulator of Cd ($NBRs_{Cd}$ of 1.42 and 1.84, respectively) and, in CF, also of Pb ($NBRs_{Pb}$ of 1.42). Hence, the cultivation of the *Virgo* genotype could be a good choice for enhancing the safety of rice in Countries where soils and/or irrigation waters are exclusively contaminated by high amounts of As, whereas the contemporary minimization of the three toxic elements can be achieved irrigating the genotype *Apollo* with CF, or the *CRV 390* with SA or, finally, the *Selenio* with SP irrigation. However, also other genotypes could be kept into consideration for pursuing As minimization aims in rice grain. In a previous study of the same research group (Spanu et al., 2012), 37 rice genotypes were cultivated in the same field used in this experiment and irrigated with CF or SP methods. In that research, the *Arsenal* and the *Vulcano* cultivars provided the least bioaccumulation of As (NBR_{As} of 0.58, CF, and

Table 5
Ranges of normalized bioaccumulation ratios (NBRs) for As, Cd, Cu, Mn, Mo, Ni, Pb irrigated by SP, SA and CF as a function of the rice genotype.

	Range of NBRs for CF (genotype, subspecie)	Range of NBRs for SA (genotype, subspecie)	Range of NBRs for SP (genotype, subspecie)
As	0.64 (*Virgo*, J) – 1.42 (*Carnise*, J)	0.57 (*Virgo*, J) – 1.57 (*Musa*, J)	0.54 (*Virgo*, J) -1.36 (*Opale*, J)
Cd[a]	0.44 (*Balilla*, J) – 2.14 (*Oceano*, I)	0.52 (*Balilla*, J) – 1.68 (*Oceano*, I)	0.35 (*Balilla*, J) – 1.72 (*Opale*, J)
Cu	0.60 (*Balilla*, J) – 2.46 (*CRV 108*, J)	0.86 (*Balilla*, J) – 1.12 (*Musa*, J)	0.70 (*Musa*, J) – 1.23 (*Thaibonnet*, I)
Mn	0.68 (*Luxor*, J) – 1.41 (*Salvo*, I)	0.67 (*Luxor*, J) -1.28 (*Carnise*, J)	0.29 (*CRV 04*, J) – 2.09 (*Urano*, I)
Mo	0.57 (*Balilla*, J) – 1.85 (*Salvo*, I)	0.68 (*CRV 108*, J) – 1.45 (*Salvo*, I)	0.70 (*Balilla*, J) – 1.62 (*Salvo*, I)
Ni	0.35 (*Balilla*, J) – 2.08 (*Urano*, I)	0.62 (*Balilla*, J) – 1.52 (*Oceano*, I)	0.54 (*Aleramo*, J) – 1.82 (*Urano*, I)
Pb	0.30 (*Carnaroli*, J) – 4.11 (*Salvo*, I)	0.49 (*Carnise*, J) – 2.16 (*Cerere*, J)	0.53 (*Carnise*, J) – 1.95 (*Opale*, J)
Se[a]	0.72 (*CRV 04*, J) – 1.54 (*Carnise*, J)	0.78 (*CRV 04*, J) – 1.30 (*Carnaroli*, J)	0.66 (*CRV 390*, J) – 1.52 (*Apollo*, I)
(As + Cd)[b]	0.65 (*Apollo*, I) – 1.51 (*Oceano*, I)	0.68 (*Ronaldo*, J) – 1.56 (*Musa*, J)	0.60 (*Selenio*, J) – 1.54 (*Opale*, J)
(As + Pb)[b]	0.63 (*CRV 114*, J) – 2.58 (*Salvo*, I)	0.69 (*Thaibonnet*, I) - 1.81 (*Cerere*, J)	0.65 (*Virgo*, J) – 1.65 (*Opale*, J)
(Cd + Pb)[b]	0.48 (*Balilla*, J) - 2.86 (*Salvo*, I)	0.65 (*CRV 390*, J) - 1.44 (*Cerere*, J)	0.58 (*Balilla*, J) – 1.83 (*Opale*, J)
(As + Cd + Pb)[b]	0.65 (*Apollo*, I) – 2.26 (*Salvo*, I)	0.74 (*CRV 114*, J) – 1.45 (*Cerere*, J)	0.67 (*Selenio*, J) – 1.68 (*Opale*, J)
All elements[b]	0.64 (*Apollo*, I) – 1.73 (*Salvo*, I)	0.86 (*Thaibonnet*, I) – 1.21 (*Musa*, J)	0.70 (*Balilla*, J) – 1.36 (*Urano*, I)

I, Indica genotype; J, Japonica genotype.
[a] Calculated as the average amount on three years.
[b] Averaged value among the elements considered.

0.46, SP, respectively), whereas the *Sapise* 164 and the *Ulisse* genotypes revealed the highest efficiency on As bioaccumulation (NBR_{As} of 1.44, CF, and 1.82, SP, respectively). It is important to underline that only nine genotypes were used in both studies, hence – in the whole research – 54 different genotypes were studied in relationship to their As bioaccumulation capabilities, all described in detail by data reported either in Tables 2S–7S as well as in Spanu et al. (2012).

Another problem of utmost concern worldwide is the bioaccumulation of Cd in rice grain. In the past, literature studies stated that "*..it may, therefore, be difficult to maintain low Cd and As concentrations in grain simultaneously by means of water management alone*" (Arao et al., 2009). These results simply refute this belief, as demonstrated by data reported in Tables 2S and 4S, showing that the 70% of the rice genotypes kept into consideration in this study exhibits a contemporary reduction of the As and Cd amounts in rice grain passing from CF to SP irrigation. Since all the rice genotypes used in this experiment were commonly available and currently cultivated in Europe, although sometimes with different names, this result represents an effective improvement of that reported by Ishikawa et al. (2016), that identified in the *Koshihikari Kan No. 1* the only genotype able to contemporary minimize the amounts of As e Cd in rice grain. The aim of simultaneously minimizing at the highest level both the As and Cd amounts in kernels can be accomplished by cultivating the *Apollo* genotype in CF irrigation ($NBRs_{As+Cd}$ of 0.65) or – mainly - the *Selenio* genotype in SP irrigation ($NBRs_{As+Cd}$ of 0.60). On the contrary, the use of the SA irrigation should be discouraged in this case, due to its very high tendency to increase by several times the Cd concentration in rice grain in comparison to amounts measured using both the CF and SP methods. These observations were indirectly confirmed by the results of Hu et al. (2013a), that evidenced how intermittent irrigation methods similar to SA (Spanu et al., 2018) increase the amount of Cd in grains from seven different Chinese rice genotypes also of more than 40 times the amount normally found when irrigated by CF. The very wide variability of Cd bioaccumulation in rice grain as a function of the genotype was well described by the contribution of Shi et al., (Shi et al., 2009), where 110 rice genotypes irrigated by CF gave $NBRs_{Cd}$ ratios ranging between 0.18 and 2.59. Data obtained in this and in our previous study (Spanu et al., 2018) ($NBRs_{Cd}$ ratios between 0.48; genotype *Balilla*, and 2.14; genotype *Oceano*) are well within this range. The data reported in both these studies allow to demonstrate the constantly low Cd-bioaccumulating capabilities of the *Balilla* genotype, which is always the worst bioaccumulator of Cd ($NBRs_{Cd}$ ratio ranging between 0.35 for SP and 0.54 for SA on the three years of experimentation).

Although data for Pb are limited only to one year of experimentation, they can however afford a number of preliminary, but meaningful information. *Carnaroli* (CF, NBR_{Pb} of 0.30) and *Carnise* (SA and SP, $NBRs_{Pb}$ of 0.49 and 0.53, respectively) are the genotypes that exhibit the strongest tendency to reduce the bioaccumulation of Pb in rice grain, whereas *Salvo, Cerere* and *Opale* are the genotypes more efficient in the increasing of the elemental concentration in kernels, always with $NBRs_{Pb}$ values significantly higher than the average amount for the considered irrigation method (e.g. $NBRs_{Pb}$ of 4.11, 2.16 and 1.94 for CF, SA and SP methods, respectively). Since soils of mining sites containing high amounts of natural Pb can be concomitantly interested by quite high concentrations of As and/or Cd (Alloway, 2013; Adriano, 2001), it is worthy of interest to identify those rice genotypes able to contemporarily minimize the amounts of the Pb/As or the Pb/Cd couples of elements. Again, *Virgo* is the best genotype able to simultaneously minimize the concentration of As and Pb when SP- irrigated, while the same results were achieved SA irrigation on *Ronaldo* and CF on *CRV 108* genotype; in all these scenarios the $NBRs_{As+Pb}$ values are always below 0.70. As for the simultaneous minimization of Cd and Pb, again *Balilla* genotype represents the best choice when CF and SP irrigation are employed ($NBRs_{Cd+Pb}$ values of 0.48 and 0.58, respectively), while the best results using SA irrigation were obtained by the *CRV 390* genotype ($NBRs_{Cd+Pb}$ of 0.65). Finally, genotypes *Apollo* ($NBRs_{As+Cd+Pb}$ of 0.65), *CRV 114* ($NBRs_{As+Cd+Pb}$ of 0.74), and *Selenio* ($NBRs_{As+Cd+Pb}$ of 0.67) are those most effective in the contemporary reduction of these toxic elements in CF, SA and SP irrigation methods, respectively.

Data reported in Tables 5S-7S may allow to make the right choice in order to tune the amounts of one or more oligoelements in rice grain for reaching specific nutritional objectives. One peculiar case is given by Ni, because it is the only element whose bioaccumulation has been always observed when SP irrigation is used. Given its high allergenic power, the need to minimize its concentration along with one of the cited toxic elements might be a desirable aim. In this case, the *Balilla* genotype represents the best choice ($NBRs_{As+Cd+Pb+Ni} = 0.67$), but also *Orione, Selenio* and *Virgo* genotypes can represent valuable possibilities, since they all present $NBRs_{As+Cd+Pb+Ni}$ values below 0.75.

A constant behavior in the elemental bioaccumulation capabilities of rice grain for a small number of genotypes can be seen in Table 5. Beyond that for Cd in all irrigation methods, the *Balilla* genotype minimizes the bioaccumulation of Ni and Cu (CF and SA) and of Mo (CF and SP), as well as it happens for *Virgo* genotype for As in all irrigation methods. *CRV 04* minimizes the bioaccumulation of Se in CF and SA, whereas *Luxor* does the same for Mn. Conversely, few genotypes exhibit wide capabilities to bioaccumulate high amounts of the elements in exam. *Salvo* always prevails as the best bioaccumulator of Mo in all irrigation methods, as well as for Mn and Pb in CF, whereas *Opale* maximizes the amount of As, Cd and Pb in SP irrigation. Furthermore, the *Urano* genotype is the best bioaccumulator of Ni and Mn in SP, but the same happens also for Ni in CF. In addition, the *Oceano* cultivar highly bioconcentrates Cd using CF and – mainly - SA irrigation, as well as it happens also for Ni in SA irrigation. Finally, few genotypes exhibit a mixed behavior: among others, *Carnise* is a bad bioaccumulator for Pb in SA and SP, but is the best bioaccumulator for Mn in SA as well as for As and Se in CF.

Finally, it is worthy to note that, despite the fact that the rice genotypes belonging to the Indica subspecies are less than 25% of the total considered in this study, in more than 50% of the 24 couples element/irrigation method they exhibit the best bioaccumulation capabilities. On the other hand, no Indica genotype has resulted to be effective in minimizing the bioaccumulation of any element. Hence, keeping into consideration also the evidences from the PCA analysis, it can be concluded that the six genotypes belonging to the Indica subspecies demonstrated a clear tendency to be good bioaccumulators of the eight analytes considered in this study, and that this behavior is more definite (as demonstrated by a greater grouping in the score plot of PCA, and an higher number of best bioaccumulating genotypes as reported in Table 5) when they were irrigated by means of CF or SP methods rather than in SA method.

4. Conclusions

For the first time the concentration of eight elements of real or potential environmental concern (As, Pb, Cd, Cu, Mn, Mo, Ni and Se) has been measured by an original and validated ICP-MS method in rice grains from 26 genotypes cultivated in the same site and irrigated with the same water, with the only difference in the irrigation method used (i.e. CF, SA, SP). Data obtained show that the bioaccumulation of the elements in kernels is primarily affected by the nature of the irrigation method, and the adoption of intermittent methods like SA or SP is able to modify the typical tendency of bioaccumulation exhibited for each element in rice irrigated by means CF.

Although further experimental work is needed, the outcomes of this study should be considered as promising. First of all, a proper combination of irrigation method and rice genotype has proved to be able to achieve specific results on food safety, concerning the minimization of the amounts of one or more toxic elements in kernels. The contemporary minimization of the amounts of all toxic elements is possible irrigating by SP a wide number of different rice genotypes. Moreover, our results push towards the planning of rice cultivations able to customize the amount of selected oligoelements according to the specific needs of people.

CRediT authorship contribution statement

Antonino Spanu: Conceptualization, Methodology, Writing - review & editing, Supervision. **Massimiliano Valente:** Investigation. **Ilaria Langasco:** Investigation, Validation. **Riccardo Leardi:** Formal analysis, Writing - review & editing. **Anna Maria Orlandoni:** Data curation. **Marco Ciulu:** Formal analysis, Writing - review & editing. **Mario Antonello Deroma:** Investigation. **Nadia Spano:** Writing - review & editing. **Francesco Barracu:** Investigation. **Maria I. Pilo:** Writing - review & editing. **Gavino Sanna:** Methodology, Writing - original draft, Writing - review & editing, Validation, Supervision.

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.

Acknowledgements

The authors gratefully thank the *CeSAR UNISS, Centro Servizi di Ateneo per la Ricerca* of the University of Sassari, for access to the ICP-MS instrument, and the University of Sassari, that has been partly financed this study by means the "Fondo di Ateneo per la Ricerca 2019".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.142484.

References

Adriano, D.C., 2001. Trace elements in terrestrial environments. Biogeochemistry, Bioavailability, and Risks of Metals, Springer, New York https://doi.org/10.1007/978-0-387-21510-5.
Ahmed, Z.U., Panaullah, G.M., Gauch Jr., H., McCouch, S.R., Tyagi, W., Kabir, M.S., Duxbury, J.M., 2010. Genotype and environment effects on rice (Oryza Sativa L.) grain arsenic concentration in Bangladesh. Plant Soil 338 (1–2), 367–382. https://doi.org/10.1007/s11104-010-0551-7.
Ali, W., Mao, K., Zhang, H., Junaid, M., Xu, N., Rasool, A., Feng, X., Yang, Z., 2020. Comprehensive review of the basic chemical behaviours, sources, processes, and endpoints of trace element contamination in paddy soil-rice systems in rice-growing countries. J. Hazard. Mater. 397, 122720. https://doi.org/10.1016/j.jhazmat.2020.122720.
Alloway B. (ed). Heavy Metals in Soils. Environmental Pollution, vol 22. 2013, Springer, Dordrecht. DOI: doi:https://doi.org/10.1007/978-94-007-4470-7.
American Academy of Dermatology. Position Statement on Nickel Sensitivity, Approval by the Board of Directors on August 22, 2015. Available on the web at https://server.aad.org/Forms/Policies/Uploads/PS/PS-Nickel%20Sensitivity.pdf (last access: 24 June 2020).
APAT-IRSA CNR Metodi Analitici per le Acque; Belli, M., Centioli, D., Zorzi, P., Umberto, S., Capri, S., Pagnotta, R., Pettine, M., Eds.; APAT: Rome, Italy, 2003; ISBN 88-448-0083-7 (in Italian language). Available on the web at http://www.irsa.cnr.it/Metodi.html (last access: 24 June 2020).
Arao, T., Kawasaki, A., Baba, K., Mori, S., Matsumoto, S., 2009. Effects of water management on cadmium and arsenic accumulation and dimethylarsinic acid concentrations in Japanese rice. Environ. Sci. Technol. 43, 9361–9367. https://doi.org/10.1021/es9022738.
Ashraf, U., Hussain, S., Akbar, N., Anjum, S.A., Hassan, W., Tang, X., 2018. Water management regimes alter Pb uptake and translocation in fragrant Rice. Ecotoxicol. Environ. Saf. 149, 128–134. https://doi.org/10.1016/j.ecoenv.2017.11.033.
Bakhat, H.F., Zia, Z., Fahad, S., Abbas, S., Hammad, H.M., Shahzad, A.N., Abbas, F., Alharby, H., Shahid, M., 2017. Arsenic uptake, accumulation and toxicity in rice plants: possible remedies for its detoxification: a review. Environ. Sci. Pollut. Res. 24 (10), 9142–9158. https://doi.org/10.1007/s11356-017-8462-2.
Barceloux, D.G., Barceloux, D., 1999. Molybdenum. J. Toxicol. Clin. Toxicol. 37 (2), 231–237. https://doi.org/10.1081/clt-100102422.
Becker, J.S., 2008. Inorganic Mass Spectrometry: Principles and Applications. John Wiley & Sons, Ltd. Chichester, UK (ISBN: 9780470012000).
Birke, M., Reimann, C., Demetriades, A., Rauch, U., Lorenz, H., Harazim, B., Glatte, W., 2010. Determination of major and trace elements in European bottled mineral water — analytical methods. J. Geochem. Explor. 107 (3), 217–226. https://doi.org/10.1016/j.gexplo.2010.05.005.
Brewer, G.J., 2012. Copper excess, zinc deficiency, and cognition loss in Alzheimer's disease. BioFactors 38 (2), 107–113. https://doi.org/10.1002/biof.1005.
Brown, P.H., Welch, R.M., Cary, E.E., 1987. Nickel: a micronutrient essential for higher plants. Plant Physiol. 85 (3), 801–803. https://doi.org/10.1104/pp.85.3.801.
Campbell, L.C., Nable, R.O., 1988. Physiological functions of Manganese in plants. In Manganese in Soils and Plants; Springer Netherlands, 139–154 https://doi.org/10.1007/978-94-009-2817-6_11.
Canfield, R.L., Henderson Jr., C.R., Cory-Slechta, D.A., Cox, C., Jusko, T.A., Lanphear, B.P., 2003. Intellectual impairment in children with blood lead concentrations below 10 mg per deciliter. N. Engl. J. Med. 348 (16), 1517–1526. https://doi.org/10.1056/nejmoa022848.
Cao, Z.H., Hu, Z.Y., 2000. Copper contamination in paddy soils irrigated with wastewater. Chemosphere 41 (1–2), 3–6. https://doi.org/10.1016/s0045-6535(99)00383-5.
Cao, Z.H., Wang, X.C., Yao, D.H., Zhang, X.L., Wong, M.H., 2001. Selenium geochemistry of paddy soils in Yangtze River Delta. Environ. Int. 26 (5–6), 335–339. https://doi.org/10.1016/s0160-4120(01)00009-5.
Cersosimo, M.G., Koller, W.C., 2006. The diagnosis of manganese-induced parkinsonism. NeuroToxicology 27 (3), 340–346. https://doi.org/10.1016/j.neuro.2005.10.006.
Chaney, R.L., Angle, J.S., Broadhurst, C.L., Peters, C.A., Tappero, R.V., Sparks, D.L., 2007. Improved understanding of hyperaccumulation yields commercial phytoextraction and phytomining technologies. J. Environ. Qual. 36 (5), 1429–1443. https://doi.org/10.2134/jeq2006.0514.
Chowdhury, M.T.A., Deacon, C.M., Jones, G.D., Imamul Huq, S.M., Williams, P.N., Manzurul Hoque, A.F.M., Winkel, L.H.E., Price, A.H., Norton, G.J., Meharg, A.A., 2017. Arsenic in Bangladeshi soils related to physiographic region, paddy management, and micro- and macro-elemental status. Sci. Total Environ. 590–591, 406–415. https://doi.org/10.1016/j.scitotenv.2016.11.191.
Clemens, S., Ma, J.F., 2016. Toxic Heavy metal and metalloid accumulation in crop plants and foods. Annu. Rev. Plant Biol. 67 (1), 489–512. https://doi.org/10.1146/annurev-arplant-043015-112301.
Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union, 20.12.2006, L 364/5 EN. Available on the web at https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32006R1881&from=EN (last access: 24 June 2020).
Commission Regulation (EU) 2015/1006 of 25 June 2015, amending Regulation (EC) No 1881/2006 as regards maximum levels of inorganic arsenic in foodstuffs. Official Journal of the European Union, 26.6.2015, L 161/14 EN. Available on the web at https://

eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32015R1006&from=EN (last access: 24 June 2020).
Currie, L.A., 1999. Nomenclature in evaluation of analytical methods including detection and quantification capabilities. Anal. Chim. Acta 391 (2), 105–126. https://doi.org/10.1016/s0003-2670(99)00104-x.
J.M. Duxbury and G. Panaullah, "Remediation of As for agriculture sustainability, food security and health in Bangladesh". FAO Water Working Paper, FAO, Rome, 2007. Available on the web at http://www.fao.org/3/a-ap102e.pdf (last access: 24 June 2020).
Fangmin, C., Ningchun, Z., Haiming, X., Yi, L., Wenfang, Z., Zhiwei, Z., Mingxue, C., 2006. Cadmium and lead contamination in japonica rice grains and its variation among the different locations in Southeast China. Sci. Total Environ. 359 (1–3), 156–166. https://doi.org/10.1016/j.scitotenv.2005.05.005.
FAO. Rice Information; FAO: Rome, Italy, 2002; Vol. 3. Available in the web at http://www.fao.org/3/y4347e/y4347e00.htm (last access: 24 June 2020).
FAO. World reference base for soil resources 2014. Update 2015. Rome, 2015. Available at the web on http://www.fao.org/3/i3794en/I3794en.pdf (last access: 24 June 2020).
FAO (Food Agriculture Organization UN), WHO (World Health Organization). 2010. Evaluation of certain food additives and contaminants: seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives. WHO Tech. Report Ser. 960, WHO, Geneva, Switzerland. Available on the web at http://whqlibdoc.who.int/trs/WHO_TRS_960_eng.pdf (last access: 24 June 2020).
FAO-WHO Codex Alimentarius, Codex Alimentarius Commission, 37th Session, Geneva, Switzerland, 14–18 July 2014. Report of the eighth session of the Codex Committee on contaminants in foods. The Hague, The Netherlands, 31 March – 4 April 2014. Available on the web at http://www.codexalimentarius.org/download/report/906/REP14_CFe.pdf (last access: 24 June 2020).
FAO-WHO Codex Alimentarius, General Standard for Contaminants and Toxins in Food and Feed, CXS 193-1995. Available on the web at http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXS%2B193-1995%252FCXS_193e.pdf (last access: 24 June 2020).
Frazzoli, C., D'Amato, M., Caroli, S., Zray, G., 2006. Arsenic and Other Potentially Toxic Trace Elements in Rice. The Determination of Chemical Elements in Food. John Wiley & Sons, Inc, pp. 383–400 https://doi.org/10.1002/9780470141007.ch12.
Fu, J., Zhou, Q., Liu, J., Liu, W., Wang, T., Zhang, Q., Jiang, G., 2008. High levels of heavy metals in rice (Oryzasativa L.) from a typical E-waste recycling area in Southeast China and its potential risk to human health. Chemosphere 71 (7), 1269–1275. https://doi.org/10.1016/j.chemosphere.2007.11.065.
Grant, C.A., Clarke, J.M., Duguid, S., Chaney, R.L., 2008. Selection and breeding of plant cultivars to minimize cadmium accumulation. Sci. Total Environ. 390 (2–3), 301–310. https://doi.org/10.1016/j.scitotenv.2007.10.038.
Halder, D., Saha, J.K., Biswas, A., 2020. Accumulation of essential and non-essential trace elements in rice grain: possible health impacts on rice consumers in West Bengal. India. Science of The Total Environment 706, 135944. https://doi.org/10.1016/j.scitotenv.2019.135944.
Hatfield, D.L., Tsuji, P.A., Carlson, B.A., Gladyshev, V.N., 2014. Selenium and Selenocysteine: roles in Cancer, health, and development. Trends Biochem. Sci. 39 (3), 112–120. https://doi.org/10.1016/j.tibs.2013.12.007.
Honma, T., Ohba, H., Kaneko-Kadokura, A., Makino, T., Nakamura, K., Katou, H., 2016. Optimal soil eh, pH, and water management for simultaneously minimizing arsenic and cadmium concentrations in rice grains. Environmental Science & Technology 50 (8), 4178–4185. https://doi.org/10.1021/acs.est.5b05424.
Horwitz, W., 1982. Evaluation of analytical methods used for regulation of foods and drugs. Anal. Chem. 54 (1), 67–76. https://doi.org/10.1021/ac00238a002.
Hu, P., Huang, J., Ouyang, Y., Wu, L., Song, J., Wang, S., Li, Z., Han, C., Zhou, L., Huang, Y., Luo, Y., Christie, P., 2013a. Water management affects arsenic and cadmium accumulation in different rice cultivars. Environ. Geochem. Health 35 (6), 767–778. https://doi.org/10.1007/s10653-013-9533-z.
Hu, P., Li, Z., Yuan, C., Ouyang, Y., Zhou, L., Huang, J., Huang, Y., Luo, Y., Christie, P., Wu, L., 2013b. Effect of water management on cadmium and arsenic accumulation by rice (Oryza sativa L.) with different metal accumulation capacities. J. Soils Sediments 13, 916–924. https://doi.org/10.1007/s11368-013-0658-6.
Hu, Y., Cheng, H., Tao, S., 2016. The challenges and solutions for cadmium-contaminated rice in China: a critical review. Environ. Int. 92–93, 515–532. https://doi.org/10.1016/j.envint.2016.04.042.
IARC (2012). "Nickel and nickel compounds" Archived September 20, 2017, at the Wayback Machine in IARC Monogr Eval Carcinog Risks Hum. Volume 100C. pp. 169–218. Available on the web at https://monographs.iarc.fr/wp-content/uploads/2018/06/mono100C-10.pdf (last access: 24 June 2020).
Ishikawa, S., Makino, T., Ito, M., Harada, K., Nakada, H., Nishida, I., Nishimura, M., Tokunaga, T., Shirao, K., Yoshizawa, C., Matsuyama, M., Abe, T., Arao, T., 2016. Low-cadmium Rice (Oryza sativa L.) cultivar can simultaneously reduce arsenic and cadmium concentrations in rice grains. Soil Science and Plant Nutrition 62 (4), 327–339. https://doi.org/10.1080/00380768.2016.1144452.
Islam, S., Rahman, M.M., Islam, M.R., Naidu, R., 2016. Arsenic accumulation in rice: consequences of rice genotypes and management practices to reduce human health risk. Environ. Int. 96, 139–155. https://doi.org/10.1016/j.envint.2016.09.006.
Japan External Trade Organization, Specifications and Standards for Foods, Food Additives, etc. Under the Food Sanitation Act. (Abstract) 2010, April 2011. Available on the web at https://www.jetro.go.jp/ext_images/en/reports/regulations/pdf/foodext2010e.pdf (last access: 24 June 2020).
Jo, G., Todorov, T.I., 2019. Distribution of nutrient and toxic elements in brown and polished rice. Food Chem. 289, 299–307. https://doi.org/10.1016/j.foodchem.2019.03.040.
Jorhem, L., Åstrand, C., Sundström, B., Baxter, M., Stokes, P., Lewis, J., Grawé, K.P., 2008a. Elements in Rice from the Swedish market: 1. Cadmium, lead and arsenic (total and inorganic). Food Additives & Contaminants: Part A 25 (3), 284–292. https://doi.org/10.1080/02652030701474219.
Jorhem, L., Åstrand, C., Sundström, B., Baxter, M., Stokes, P., Lewis, J., Grawé, K.P., 2008b. Elements in rice on the Swedish market: part 2. Chromium, copper, Iron, manganese, platinum, rubidium, selenium and zinc. Food Additives & Contaminants: Part A 25 (7), 841–850. https://doi.org/10.1080/02652030701701058.
Kabata-Pendias, A.; Mukherjee, A. B. Trace Elements from Soil to Human; Springer Berlin Heidelberg, 2007. doi:10.1007/978-3-540-32714-1.
Kawada, T., Suzuki, S., 1998. A review on the cadmium content of rice, daily cadmium intake, and accumulation in the kidneys. J. Occup. Health 40 (4), 264–269. https://doi.org/10.1539/joh.40.264.
Kirkham, M.B., 2006. Cadmium in plants on polluted soils: effects of soil factors, hyperaccumulation, and amendments. Geoderma 137 (1–2), 19–32. https://doi.org/10.1016/j.geoderma.2006.08.024.
Leardi, R.; Melzi, C.; Polotti, G. CAT (Chemometric Agile Tool). Available and freely downloadable at the web on http://gruppochemiometria.it/index.php/software (last access: 24 June 2020).
Li, Y., Trush, M.A., Yager, J.D., 1994. DNA damage caused by reactive oxygen species originating from a copper-dependent oxidation of the 2-Hydroxy catechol of estradiol. Carcinogenesis 15 (7), 1421–1427. https://doi.org/10.1093/carcin/15.7.1421.
Li, H., Luo, N., Li, Y.W., Cai, Q.Y., Li, H.Y., Mo, C.H., Wong, M.H., 2017. Cadmium in Rice: transport mechanisms, influencing factors, and minimizing measures. Environ. Pollut. 224, 622–630. https://doi.org/10.1016/j.envpol.2017.01.087.
Li, X., Peng, P., Long, J., Dong, X., Jiang, K., Hou, H., 2020. Plant-induced insoluble cd mobilization and Cd redistribution among different rice cultivars. J. Clean. Prod. 256, 120494. https://doi.org/10.1016/j.jclepro.2020.120494.
Liao, L.; Xu, J.; Peng, S.; Qiao, Z.; Gao, X. Uptake and Bioaccumulation of Heavy Metals in Rice Plants as Affect by Water Saving Irrigation. Advance Journal of Food Science and Technology 2013, 5 (9), 1244–1248. doi:10.19026/ajfst.5.3090.
Liu, J., Li, K., Xu, J., Zhang, Z., Ma, T., Lu, X., Yang, J., Zhu, Q., 2003. Lead toxicity, uptake, and translocation in different Rice cultivars. Plant Sci. 165 (4), 793–802. https://doi.org/10.1016/s0168-9452(03)00273-5.
Liu, J., Ma, X., Wang, M., Sun, X., 2013. Genotypic differences among rice cultivars in Lead accumulation and translocation and the relation with grain Pb levels. Ecotoxicol. Environ. Saf. 90, 35–40. https://doi.org/10.1016/j.ecoenv.2012.12.007.
Malagoli, M.; Schiavon, M.; dall'Acqua, S.; Pilon-Smits, E. A. H. Effects of Selenium Biofortification on Crop Nutritional Quality. Front. Plant Sci. 2015, 6. doi:https://doi.org/10.3389/fpls.2015.00280.
Marin, A.R., Masscheleyn, P.H., Patrick Jr., W.H., 1993. Soil redox-pH stability of arsenic species and its influence on arsenic uptake by rice. Plant Soil 152 (2), 245–253. https://doi.org/10.1007/bf00029094.
McLaughlin, M., Parker, D., Clarke, J., 1999. Metals and micronutrients – food safety issues. Field Crop Res. 60 (1–2), 143–163. https://doi.org/10.1016/s0378-4290(98)00137-3.
Meharg, C., Meharg, A.A., 2015. Silicon, the silver bullet for mitigating biotic and abiotic stress, and improving grain quality, in Rice? Environ. Exp. Bot. 120, 8–17. https://doi.org/10.1016/j.envexpbot.2015.07.001.
Meharg, A.A., Rahman, M.M., 2003. Arsenic contamination of Bangladesh paddy field soils: implications for rice contribution to arsenic consumption. Environmental Science & Technology 37 (2), 229–234. https://doi.org/10.1021/es0259842.
Meharg, A.A., Williams, P.N., Adomako, E., Lawgali, Y.Y., Deacon, C., Villada, A., Cambell, R.C.J., Sun, G., Zhu, Y.-G., Feldmann, J., Raab, A., Zhao, F.-J., Islam, R., Hossain, S., Yanai, J., 2009. Geographical variation in total and inorganic arsenic content of polished (white) Rice. Environmental Science & Technology 43 (5), 1612–1617. https://doi.org/10.1021/es802612a.
Mendel, R.R., 2011. Cell biology of molybdenum in plants. Plant Cell Rep. 30 (10), 1787–1797. https://doi.org/10.1007/s00299-011-1100-4.
Mitra, A., Chatterjee, S., Moogouei, R., Gupta, D., 2017. Arsenic accumulation in rice and probable mitigation approaches: a review. Agronomy 7 (4), 67. https://doi.org/10.3390/agronomy7040067.
Miura, K., Ashikari, M., Matsuoka, M., 2011. The role of QTLs in the breeding of high-yielding rice. Trends Plant Sci. 16 (6), 319–326. https://doi.org/10.1016/j.tplants.2011.02.009.
Moreno-Jiménez, E., Meharg, A.A., Smolders, E., Manzano, R., Becerra, D., Sánchez-Llerena, J., Albarrán, A., López-Piñero, A., 2014. Sprinkler irrigation of rice fields reduces grain arsenic but enhances cadmium. Sci. Total Environ. 485-486, 468–473. https://doi.org/10.1016/j.scitotenv.2014.03.106.
Norton, G.J., Islam, M.R., Deacon, C.M., Zhao, F.-J., Stroud, J.L., McGrath, S.P., Islam, S., Jahiruddin, M., Feldmann, J., Price, A.H., et al., 2009. Identification of low inorganic and total grain arsenic rice cultivars from Bangladesh. Environmental Science & Technology 43 (15), 6070–6075. https://doi.org/10.1021/es901121j.
Norton, G.J., Williams, P.N., Adomako, E.E., Price, A.H., Zhu, Y., Zhao, F.-J., McGrath, S., Deacon, C.M., Villada, A., Sommella, A., Lu, Y., Ming, L., Mangala, P., De Silva, C.S., Brammer, H., Dasgupta, T., Islam, M.R., Meharg, A.A., 2014. Lead in Rice: analysis of baseline lead levels in market and field collected rice grains. Sci. Total Environ. 485–486, 428–434. https://doi.org/10.1016/j.scitotenv.2014.03.090.
Norton, G.J., Deacon, C.M., Mestrot, A., Feldmann, J., Jenkins, P., Baskaran, C., Meharg, A.A., 2015. Cadmium and Lead in vegetable and fruit produce selected from specific regional areas of the UK. Sci. Total Environ. 533, 520–527. https://doi.org/10.1016/j.scitotenv.2015.06.130.
Orasen, G., De Nisi, P., Lucchini, G., Abruzzese, A., Pesenti, M., Maghrebi, M., Kumar, A., Nocito, F.F., Baldoni, E., Morgutti, S., et al., 2019. Continuous flooding or alternate wetting and drying differently affect the accumulation of health-promoting phytochemicals and minerals in rice brown grain. Agronomy 9 (10), 628. https://doi.org/10.3390/agronomy9100628.
Pan, Y., Bonten, L.T.C., Koopmans, G.F., Song, J., Luo, Y., Temminghoff, E.J.M., Comans, R.N.J., 2016. Solubility of trace metals in two contaminated paddy soils exposed to

alternating flooding and drainage. Geoderma 261, 59–69. https://doi.org/10.1016/j.geoderma.2015.07.011.
Pansu, M.; Gautheyrou, J. Handbook of Soil Analysis. Mineralogical, Organic and Inorganic Methods; Springer: Berlin, Germany, 2006; pp. 581–591. ISBN 9783540312116.
Parfitt, J. M. B.; Concenço, G.; Scivittaro, W. B.; Andres, A.; da Silva, J. T.; Pinto, M. A. B. Soil and Water Management for Sprinkler Irrigated Rice in Southern Brazil. In Advances in International Rice Research; InTech, 2017. doi:https://doi.org/10.5772/66024.
Phuong, T.D., Kokot, S., Chuong, P.V., Tong Khiem, D., 1999. Elemental content of Vietnamese rice. Part 1. Sampling, analysis and comparison with previous studies. Analyst 124 (4), 553–560. https://doi.org/10.1039/a808796b.
Popular Republic of China, national standard of China on Maximum Levels of Contaminants in Foods, 25.01.2005. Available on the web at https://apps.fas.usda.gov/gainfiles/200608/146208660.pdf (last access: 24 June 2020).
Qian, Y., Chen, C., Zhang, Q., Li, Y., Chen, Z., Li, M., 2010. Concentrations of cadmium, Lead, mercury and arsenic in Chinese market milled rice and associated population health risk. Food Control 21 (12), 1757–1763. https://doi.org/10.1016/j.foodcont.2010.08.005.
Rahman, M.A., Rahman, M.M., Reichman, S.M., Lim, R.P., Naidu, R., 2014. Heavy metals in australian grown and imported rice and vegetables on sale in Australia: health hazard. Ecotoxicol. Environ. Saf. 100, 53–60. https://doi.org/10.1016/j.ecoenv.2013.11.024.
Reddy, C.N., Patrick Jr., W.H., 1977. Effect of redox potential and pH on the uptake of cadmium and lead by rice plants. J. Environ. Qual. 6 (3), 259–262. https://doi.org/10.2134/jeq1977.00472425000600030005x.
Rizwan, M., Ali, S., Adrees, M., Rizvi, H., Zia-ur-Rehman, M., Hannan, F., Qayyum, M.F., Hafeez, F., Ok, Y.S., 2016. Cadmium stress in rice: toxic effects, tolerance mechanisms, and management: a critical review. Environ. Sci. Pollut. Res. 23 (18), 17859–17879. https://doi.org/10.1007/s11356-016-6436-4.
Roberts, E.A., Schilsky, M.L., 2008. Diagnosis and treatment of Wilson disease: an update. Hepatology 47 (6), 2089–2111. https://doi.org/10.1002/hep.22261.
Saifullah; Dahlawi, S.; Naeem, A.; Iqbal, M.; Farooq, M. A.; Bibi, S.; Rengel, Z. Opportunities and Challenges in the Use of Mineral Nutrition for Minimizing Arsenic Toxicity and Accumulation in Rice: A Critical Review. Chemosphere 2018, 194, 171–188. doi: doi:https://doi.org/10.1016/j.chemosphere.2017.11.149.
Sebastian, A., Prasad, M.N.V., 2013. Cadmium minimization in rice. A review. Agronomy for Sustainable Development 34 (1), 155–173. https://doi.org/10.1007/s13593-013-0152-y.
Servizio Informativo Agrario Nazionale (SIAN). Registro della varietà di specie agrarie. 2018, 252–266 (in Italian language). Available on the web at https://www.sian.it/downloadpub/zfadlx010?id=8044 (last access: 24 June 2020).
Shi, J., Li, L., Pan, G., 2009. Variation of grain Cd and Zn concentrations of 110 hybrid rice cultivars grown in a low-Cd paddy soil. J. Environ. Sci. 21 (2), 168–172. https://doi.org/10.1016/s1001-0742(08)62246-9.
da Silva, J. T.; Paniz, F. P.; Sanchez, F. e S.; Pedron, T.; Torres, D. P.; da Rocha Concenço, F. I. G.; Barbat Parfitt, J. M.; Batista, B. L. Selected Soil Water Tensions at Phenological Phases and Mineral Content of Trace Elements in Rice Grains – Mitigating Arsenic by Water Management. Agric. Water Manag. 2020, 228, 105884. doi:https://doi.org/10.1016/j.agwat.2019.105884.
Spanu, A., Pruneddu, G., Deidda, M. Primi risultati sulla coltivazione del riso (*Oryza sativa* L.) irrigato per aspersione. Rivista di Agronomia, 1989, 23, 378–384 (in Italian language). Available on the web at http://eprints.uniss.it/4634/ (last access: 24 June 2020).
Spanu, A., Murtas, A., Ledda, L., Ballone, F. Innovative agronomic techniques for rice cultivation. Conference proceedings of challenger and opportunities for sustainable rice-based production systems, Torino, Italy, 2004a, 207–216. Available on the web at https://www.researchgate.net/publication/264874823_CHALLENGES_AND_OPPORTUNITIES_FOR_SUSTAINABLE_RICE-BASED_PRODUCTION_SYSTEMS (last access: 24 June 2020).
Spanu, A.; Murtas, A.; Ledda, L.; Ballone, F. "Root system development in flooded and sprinkler irrigated rice cultivars". Conference proceedings of "Challenges and opportunities for sustainable rice-based production systems", Torino, Italy, 217–225, 2004b. Available at the web on https://www.researchgate.net/publication/264874823_CHALLENGES_AND_OPPORTUNITIES_FOR_SUSTAINABLE_RICE-BASED_PRODUCTION_SYSTEMS (last access: 24 June 2020).
Spanu, A., Murtas, A.M.L., Ballone, F., 2009. Water use and crop coefficients in sprinkler irrigated rice. Ital. J. Agron. 4, 47–58. https://doi.org/10.4081/ija.2009.2.47.
Spanu, A., Daga, L., Orlandoni, A.M., Sanna, G., 2012. The role of irrigation techniques in arsenic bioaccumulation in rice (Oryza sativa L.). Environ. Sci. Technol. 46, 8333–8340. https://doi.org/10.1021/es300636d.
Spanu, A., Valente, M., Langasco, I., Barracu, F., Orlandoni, A., 2018. M.; Sanna, G. sprinkler irrigation is effective in reducing cadmium concentration in Rice (Oryza Sativa L.) grain: a new twist on an old tale? Sci. Total Environ. 628–629, 1567–1581. https://doi.org/10.1016/j.scitotenv.2018.02.157.
Spanu, A., Langasco, I., Valente, M., Deroma, M.A., Spano, N., Barracu, F., Pilo, M.I., Sanna, G., 2020. Tuning of the amount of se in rice (Oryza sativa) grain by varying the nature of the irrigation method: development of an ICP-MS analytical protocol, validation and application to 26 different rice genotypes. Molecules 25, 1–17. https://doi.org/10.3390/molecules25081861 1861.
Srivastava, S., Suprasanna, P., D'Souza, S.F., 2012. Mechanisms of arsenic tolerance and detoxification in plants and their application in transgenic technology: a critical appraisal. International Journal of Phytoremediation 14 (5), 506–517. https://doi.org/10.1080/15226514.2011.604690.
Suda, A., Makino, T., 2016. Functional effects of manganese and iron oxides on the dynamics of trace elements in soils with a special focus on arsenic and cadmium: a review. Geoderma 270, 68–75. https://doi.org/10.1016/j.geoderma.2015.12.017.
Sun, L., Zheng, M., Liu, H., Peng, S., Huang, J.G., Cui, K., Nie, L., 2014. Water management practices affect arsenic and cadmium accumulation in rice grains. Sci. World J. ID 596438. https://doi.org/10.1155/2014/596438.
Suriyagoda, L.D.B., Dittert, K., Lambers, H., 2018. Arsenic in rice soils and potential agronomic mitigation strategies to reduce arsenic bioavailability: a review. Pedosphere 28 (3), 363–382. https://doi.org/10.1016/s1002-0160(18)60026-8.
Suttle, N.F., 1974. Recent studies of the copper-molybdenum antagonism. Proc. Nutr. Soc. 33 (3), 299–305. https://doi.org/10.1079/pns19740053.
Teklić, T., Lončarić, Z., Kovačević, V., Singh, B.R., 2013. Metallic trace elements in cereal grain - a review: how much metal do we eat? Food and Energy Security 2 (2), 81–95. https://doi.org/10.1002/fes3.24.
Thyssen, J.P., Linneberg, A., Menné, T., Johansen, J.D., 2007. The epidemiology of contact allergy in the general population – prevalence and main findings. Contact Dermatitis 57 (5), 287–299. https://doi.org/10.1111/j.1600-0536.2007.01220.x.
Tripathi, R.D., Tripathi, P., Dwivedi, S., Kumar, A., Mishra, A., Chauhan, P.S., Norton, G.J., Nautiyal, C.S., 2014. Roles for root Iron plaque in sequestration and uptake of heavy metals and metalloids in aquatic and wetland plants. Metallomics 6 (10), 1789–1800. https://doi.org/10.1039/c4mt00111g.
Tsukahara, T., Ezaki, T., Moriguchi, J., Furuki, K., Shimbo, S., Matsuda-Inoguchi, N., Ikeda, M., 2003. Rice as the most influential source of cadmium intake among general Japanese population. Sci. Total Environ. 305 (1–3), 41–51. https://doi.org/10.1016/s0048-9697(02)00475-8.
Gazzetta Ufficiale Serie Generale, 1999, n. 248, 21 October 1999. Suppl. Ordinario n. 185. Metodi Ufficiali di Analisi Chimica del Suolo (in Italian language). Available on the web at https://www.gazzettaufficiale.it/eli/gu/1999/10/21/248/so/185/sg/pdf (last access: 24 June 2020).
United Nations, Department of Economic and Social Affairs, Population Division (2015). World Population Prospects: The 2015 Revision, Key Findings and Advance Tables. Working Paper No. ESA/P/WP.241. Available on the web at https://population.un.org/wpp/publications/files/key_findings_wpp_2015.pdf (last access: 24 June 2020).
Upadhyay, M.K., Yadav, P., Shukla, A., Srivastava, S., 2018. Utilizing the potential of microorganisms for managing arsenic contamination: a feasible and sustainable approach. Frontiers in Environmental Science 6. https://doi.org/10.3389/fenvs.2018.00024.
Uraguchi, S., Fujiwara, T., 2013. Rice breaks ground for cadmium-free cereals. Curr. Opin. Plant Biol. 16 (3), 328–334. https://doi.org/10.1016/j.pbi.2013.03.012.
US Agency for Toxic Substances and Disease Registry (ATSDR). 2017. Priority List of Hazardous Substances. Available on the web at https://www.atsdr.cdc.gov/spl/resources/2017_atsdr_substance_priority_list.html (last access: 24 June 2020).
Wang, X.C., Yan, W.D., An, Z., Lu, Q., Shi, W.M., Cao, Z.H., Wong, M.H., 2003. Status of trace elements in paddy soil and sediment in Taihu Lake region. Chemosphere 50 (6), 707–710. https://doi.org/10.1016/s0045-6535(02)00209-6.
Wang, W., Zhang, Z., Yang, G., Wang, Q., 2014. Health risk assessment of Chinese consumers to nickel via dietary intake of foodstuffs. Food Additives & Contaminants: Part A 31 (11), 1861–1871. https://doi.org/10.1080/19440049.2014.964336.
WHO, Trace elements in human nutrition and health, World Health Organization, Geneva, 1996. ISBN: 9241561734. Available in the web at http://apps.who.int/iris/bitstream/10665/37931/2/9241561734_eng.pdf?ua=1 (last access: 24 June 2020).
Williams, P.N., Lei, M., Sun, G., Huang, Q., Lu, Y., Deacon, C., Meharg, A.A., Zhu, Y.-G., 2009. Occurrence and partitioning of cadmium, arsenic and lead in mine impacted paddy rice: Hunan, China. Environmental Science & Technology 43 (3), 637–642. https://doi.org/10.1021/es802412r.
Wu, Y.-J., Zhou, H., Zou, Z.-J., Zhu, W., Yang, W.-T., Peng, P.-Q., Zeng, M., Liao, B.-H., 2016. A three-year in-situ study on the persistence of a combined amendment (limestone+sepiolite) for remedying paddy soil polluted with heavy metals. Ecotoxicol. Environ. Saf. 130, 163–170. https://doi.org/10.1016/j.ecoenv.2016.04.018.
Xu, Y., Gu, D., Li, K., Zhang, W., Zhang, H., Wang, Z., Yang, J., 2019. Response of grain quality to alternate wetting and moderate soil drying irrigation in rice. Crop Sci. 59 (3), 1261–1272. https://doi.org/10.2135/cropsci2018.11.0700.
Yruela, I., 2005. Copper in plants. Braz. J. Plant Physiol. 17 (1), 145–156. https://doi.org/10.1590/S1677-04202005000100012.
Zhang, Z., Moon, C., Watanabe, T., Shimbo, S., Ikeda, M., 1996. Lead content of rice collected from various areas in the world. Sci. Total Environ. 191 (1–2), 169–175. https://doi.org/10.1016/0048-9697(96)05257-6.
Zhao, F.-J., Wang, P., 2019. Arsenic and cadmium accumulation in rice and mitigation strategies. Plant Soil 446 (1–2), 1–21. https://doi.org/10.1007/s11104-019-04374-6.
Zhao, K., Liu, X., Xu, J., Selim, H.M., 2010. Heavy metal contaminations in a soil–rice system: identification of spatial dependence in relation to soil properties of paddy fields. J. Hazard. Mater. 181 (1–3), 778–787. https://doi.org/10.1016/j.jhazmat.2010.05.081.