

INFLUENCE OF THE NATURE OF THE IRRIGATION METHODS ON THE PHENOMENA OF BIOACCUMULATION OF EIGHT HEALTH-THREATENING ELEMENTS IN RICE GRAINS

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Abstract – Here we report results relative to the concentration, in rice grains from 26 different genotypes, of eight elements with an ascertained (As, Pb, Cd) or potential (Cu, Mn, Mo, Ni and Se) toxicity towards humans. All rice genotypes have been cultivated in the same site and irrigated with the same water using three different irrigation methods: continuous flooding, (CF), sprinkler irrigation (SP) and soil saturation (SA). The PCA analysis of results obtained demonstrate that the average amounts of almost all the elements considered - with the only exception of Ni - are minimized in rice irrigated with SP.

Keywords: rice, toxic elements, irrigation, bioaccumulation, arsenic

1. INTRODUCTION

The bioaccumulation of toxic elements in rice is of great concern worldwide. In particular, the tendency of rice to accumulate arsenic (As) [1-3] and cadmium (Cd) [4] represents a serious threat to public health, primarily in countries in which rice is the staple food [1,2,5,6]. Among the factors affecting bioconcentration phenomena of As and Cd in rice, a key role is certainly played by the nature of irrigation methods. Whereas irrigation for continuous flooding, CF (i.e. the traditional irrigation, used almost worldwide) is effective in minimizing the Cd concentration in rice grain [7,8], it may cause a dramatic increase in As concentration [7,8], reaching also concentration levels of some mg/kg. On the other hand, an opposite behavior in bioconcentration of both elements in rice grains has been frequently noted adopting water management methods other than CF (i.e. the so-called “aerobic”, “intermittent” or “oxidized” irrigations [7,8]). However, these methods are very often not satisfactorily described by researchers, and many doubts remain about how the plants were actually irrigated [9].

The sprinkler irrigation method (SP) was optimized for rice in Sardinia, Italy, [10-11] where its adoption in place of CF, applied to several rice genotypes over a number of crop years, has produced no significant differences in yields [12]. In addition, SP exhibits a number of environment-friendly features in comparison to CF: the water requirements are halved, the number of herbicide treatments needed to control weeds is greatly reduced, no preliminary leveling of soils is required,

no specific agricultural machinery (e.g., a crawler tractor) is needed. Our recent studies show that the adoption of SP cause, in comparison to data obtained from rice grain irrigated by CF, an unprecedented average reduction (ca. -98%) of the arsenic concentration measured on 37 rice genotypes [13], and – in a field experiment along two crop years – the reduction (between 13 and 28%) of the average Cd concentration in grain of 26 rice genotypes in comparison to those measured for the same genotypes irrigated with CF [9]. Surprisingly, the adoption in such experimentation of another, intermittent, water management system – i.e. the irrigation for saturation (SA) - has been substantiated an extraordinary increase of the Cd concentration (between 700 and 1000%) with respect the values in the same experiment measured for CF [9].

Beyond As and Cd, also other elements of known (or potential) health concern may bioaccumulate in rice: among others, it is worth to consider a toxic element like Pb, and oligoelements like Se, Ni, Cu, Mo and Mn, whose toxicity threshold for humans might be not too far from the upper limit of concentration range typical for rice. For these reasons, the principal aim of this contribution is to define the consequences that three different irrigation methods (i.e. CF, SP and SA) may play on the phenomena of bioaccumulation of As, Cd, Se, Ni, Cu, Mo and Mn in grain of 26 different rice genotypes cultivated in the same soil and irrigated with the same water.

2. EXPERIMENTAL

2.1. Site, Soil and Crop management

The cultivation was performed at the University of Sassari’s experimental farm, in Sardinia, Italy (39°59’N, 8°40’E; 15 m AMSL). The climate in the site chosen is Mediterranean, with scarce precipitations and high temperatures in the rice growing season (in the months from May to September over the last 50 years the area saw an average rainfall of 100.1 mm and average minimum and maximum temperatures of 15.8°C and 28.3°C, respectively). The soil is classified as a Typic Eutric Haplic Fluvisol (World Reference Base for Soil Resources). The land areas used for our study’s cultivation with CF and SA irrigation have been continuously used in this way for last 35 years. At a depth of 40 cm the soil presents a layer with very low permeability which reduces water losses by

percolation. On the other hand, the soils used for cultivating rice with SP irrigation are clayey with good water retention capacity. A total of 26 rice genotypes (20 from the Japonica subspecies and 6 from the Indica subspecies) were grown in three proximal fields of the experimental farm. All rice genotypes were cultivated in three fields (one for each irrigation method considered). The surface of each field was 3,000 m². The experimental design was a randomized block with 4 replications for each genotype. The surface of each sub-plot was 10 m². 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. Harvesting was performed between last days of September and first days of October. For each genotype and each sub-plot, yield was measured using a small plot combine harvester. For each sub-plot, 100 g of paddy rice was dried at 32°C, mechanically husked and bleached, and then analyzed.

2.2. Irrigation systems

Water used for irrigation came exclusively from Lake Omodeo, an artificial basin located in the center of Sardinia along the Tirso River.

CF: This is the most popular and used irrigation method worldwide. The ground is laser-leveled and surrounded by embankments to contain irrigation water. Plots are kept flooded (see. Fig. 1) 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 is held constantly negative, hence indicating the prevalence of reductive conditions. Each time the water level decreases to 2-3 cm additional water is provided to restore the level to 10 cm. In Italy the water consumption for cultivating rice with the continuous flooding technique ranges from 13,000 up to 65,000 m³ ha⁻¹, with an average of 20,000 m³ ha⁻¹.



Fig. 1. Continuous flooding (CF) irrigation of rice during tillering.

SA: The soil is never flooded, but it is cyclically saturated whenever the top 5 cm are dry. The amount of irrigation water provided is 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 are not required. To let the water spread homogeneously on the field, 10 cm-deep furrows are dug every 25-30 m (see Fig. 2). Water flows in the furrows and saturates the plots. By using this irrigation method, evaporation and percolation losses are minimized. The field was saturated at least once a week. In these conditions, the redox potential of soil follows a roughly cyclical behavior. Just after the saturation

of the soil it is possible to measure redox potential values very close to those measured in a continuously flooded soil. On the other hand, in the time elapsed between two consecutive saturations it is possible to measure a continuous increase of the redox potential of the soil, depending on the meteorological conditions and on the phenological phase of rice. Usually, the redox potential of the soil remains negative for the first few days after each saturation, after which it assumes positive values until the next saturation.



Fig. 2. Saturation (SA) irrigation of rice during tillering.

SP: The soil is never flooded or saturated, and the role of irrigation was to constantly keep the soil at its field capacity (i.e., when only the half the volume of the soil pores is occupied by water). Water is sprayed into the air by means of low- (or medium-) flow sprinkler heads (see Fig. 3) and letting it fall on the soil as rainfall. Hence, after sowing, 50-100 m³ ha⁻¹ of irrigation water is provided every 2-3 days, depending on meteorological conditions until the complete emergence of the rice. After this point, 200-250 m³ha⁻¹ of water were provided in the average every 4-5 days during the initial and final phases of cultivation, and every 2-3 days during the middle phase. The exact quantity of water provided in each irrigation cycle was determined by means a Class A Evaporation pan (EoA), and the value was corrected by a growing stage-specific constant *k_c* ranging from 0.4 to 1.2, depending on the phenological phase of rice [11]. The main difference between these irrigation methods consists in the oxidation state of the soil layer where the rice roots are found, which is rarely deeper than 20 cm. By using SP, the layer explored by roots is always in oxidized conditions, as demonstrated by the always positive value of the redox potential (usually

between 100 and 200 mV). Beyond the sharp reduction of water consumption, in comparison to traditional CF irrigation, the adoption of SP possesses additional agronomic advantages that are reflected in a number of key environmental advantages: the soil does not need to be leveled, crawler machinery does not need to be used, and there is a great reduction in both the number and strength of fertilization, herbicide treatments needed to control weeds and – finally - it is possible to dramatically decrease the amounts of greenhouse gas formed by paddy fields [13].



Fig. 3. Sprinkler (SP) irrigation of rice during stem elongation.

2.3. Analysis and chemometrics

Rice samples and soils were previously undergone to an acid/oxidant, microwave-assisted mineralization, whereas irrigation water was acidified prior analysis. The determination of As, Pb, Cd, Cu, Mn, Mo, Ni and Se in rice grain, in soils and in irrigation water was accomplished by means of an ICP-MS spectrometer model 7500a. Literature methods has been used to analyze soils and water [14,15], whereas a specific methods was assessed and validated in order to measure trace elements in rice grain.

Digestion of rice was performed using a basket containing ten PTFE vessels. Ca. 0.7 g of the sample was weighed on an analytical balance in a PTFE vessel and then treated with 2 cm³ of 69% HNO₃ TraceSelect[®], 1 cm³ of 30% H₂O₂ Suprapur[®] and 2 cm³ of H₂O. After closing, the vessel underwent the first mineralization step at 200 W for 10 min and then the second step at 400 W for 10 min. The vessel was then cooled in ice until room temperature before opening. Then, the solution

was quantitatively recovered, filtered off in a 0.45 µm pore size polypropylene filter and diluted with water until reaching a final volume of 20 cm³.

Analytical methods were validated in terms of LoD, LoQ and linearity (Table 1).

Table 1. LoD, LoQ and linearity parameters for the determination of trace elements in rice grain.

Element	LoD (µg/Kg)	LoQ (µg/Kg)	Linearity Range (µg/L)	Correlation coefficient R ²
As	0.16	0.53	0.05 - 500	0.9994
Cd	0.38	1.3	0.1 - 500	0.9992
Cu	0.56	1.8	1 - 500	0.9994
Mn	2.5	8.4	1 - 2000	0.9982
Mo	0.95	3.1	1 - 100	0.9992
Ni	4.0	13	1 - 100	0.9994
Pb	0.73	2.4	0.1 - 100	1.0000
Se	1.2	4.0	1 - 100	0.9998

Precision was measured in terms of repeatability (r) and intermediate precision (ip). Repeatability was measured by analyzing within the same analytical session for 5 times three different CRM samples of rice flours (i.e. NIST SRM 1568a, IRMM 804 and NCS ZC73008), whereas intermediate precision was evaluated by analyzing 3 samples of CRM rice flour 5 times in five different analytical sessions within one month. Data, reported in Table 2, are expressed in terms of CV%.

Table 2. Repeatability, r, and intermediate precision, ip, in the determination of trace elements in rice grain.

CRM	As			Cd		
	C (µg/Kg)	CV% (r)	CV% (ip)	C (µg/Kg)	CV% (r)	CV% (ip)
NCSZC 73008	102	2.9	6.6	87	0.79	5.8
NIST 1568a	290	1.8	9.4	22	4.1	2.9
IRMM 804	49	4.7	3.4	1610	3.0	3.2
CRM	Cu			Mn		
	C (µg/Kg)	CV% (r)	CV% (ip)	C (µg/Kg)	CV% (r)	CV% (ip)
NCSZC 73008	4900	0.93	3.8	17000	3.6	3.2
NIST 1568a	2400	1.7	7.6	20000	3.2	3.9
IRMM 804	2700	3.4	5.3	34000	6.6	6.5
CRM	Mo			Ni		
	C (µg/Kg)	CV% (r)	CV% (ip)	C (µg/Kg)	CV% (r)	CV% (ip)
NCSZC 73008	530	1.2	6.8	270	0.97	8.9
NIST 1568a	1460	1.5	5.5	150	0.32	8.4
IRMM 804	49	3.9	3.4	1610	0.51	3.2
CRM	Pb			Se		
	C (µg/Kg)	CV% (r)	CV% (ip)	C (µg/Kg)	CV% (r)	CV% (ip)
NCSZC 73008	80	0.35	13	61	9.8	15
NIST 1568a	10	9.8	27	38	3.5	12
IRMM 804	2700	3.0	5.3	34	9.5	6.5

n = 5

Trueness was checked with three different CRMs (i.e. NIST SRM 1568a, IRMM 804 and NCS ZC73008

rice flours). Table 3 report, for each CRM and for each element, the percentage of recovery on certified value.

Table 3. Trueness (% recovery on the certified value) in analysis of trace elements in three CRM rice flours.

CRM	As	Cd	Cu	Mn
NCSZC 73008	107	99	90	88
NIST 1568a	119	90	128	90
IRMM 804	128	nr	133	93
CRM	Mo	Ni	Pb	Se
NCSZC 73008	126	79	52	89
NIST 1568a	121	nr	nr	86
IRMM 804	nr	nr	132	87*

n = 5; nr = data not reported in the CRM analysis; * = data not certified in the CRM analysis.

Each sample was analyzed three times, and each analytical datum is the average of five replicated ICP-MS measurements.

Principal Component Analysis (PCA) of data obtained on rice samples was performed using an R-based software developed by the Group of Chemometrics of the Division of Analytical Chemistry of the Italian Chemical Society, freely downloadable from the site gruppochemiometria.it.

3. RESULTS

2.1. As, Pb, Cd, Cu, Mn, Mo, Ni and Se in rice grain

Table 4 reports the average value (on 26 genotypes) and the range of the concentration of As, Pb, Cd, Cu, Mn, Mo, Ni and Se in grain irrigated by CF, SA and SP, respectively.

Table 4. Average concentration and range of As, Pb, Cd, Cu, Mn, Mo, Ni and Se in grain from 26 rice genotypes irrigated by CF, SA and SP.

	CF (µg/Kg)		SA (µg/Kg)		SP (µg/Kg)	
	Average	Range	Average	Range	Average	Range
As	140	90-200	70	41-103	2.2	1-3
Cd	4.4	1.3-10	45	25-80	3.8	1.4-10
Cu	3000	1800-3800	3400	2900-3700	3200	2400- 900
Mn	7800	5000-11000	11500	7900-14500	800	300-1600
Mo	900	500-1600	660	470-940	360	250-560
Ni	90	40-180	270	170-400	700	400- 1500
Pb	30	7-140	19	10-30	17	7-40
Se	95	65-140	95	7-130	8	5-11

The comparison between the average concentrations of each element considered in rice grain of 26 different genotypes at varying of the water management system allow us to conclude that each element reveals a rather peculiar tendency to accumulate in rice grain as a consequence of the nature of the irrigation adopted. In addition, with the only exception of Ni, the adoption of SP irrigation cause the reduction of the average amounts of As, Se, Mo, Pb (-98%, -90%, -60% and -43% respectively) in comparison to values measured for rice irrigated with CF and of Mn, Cd, Se, (-93%, -91% and -90% respectively) with respect the values measured for rice irrigated with SA. Data obtained are also in excellent agreement with our previous studies, reporting substantial reductions of the concentration of As [13] and Se (unpublished results) in rice grain passing from CF to SP, and of the concentration of Cd [9] passing from SA to SP. On the other hand, it is evident that the adoption of SP irrigation causes the bioconcentration of Ni in rice grain (+760% in the average value, passing from CF to SP). Furthermore, the wide range of the concentrations measured for each element using a stated irrigation method give account for a substantial genotype-effect. Finally, it is possible observe that a number of rice genotypes (i.e. Balilla and Virgo) have an intrinsic low tendency to bioconcentrate elements of environmental concern, irrespectively of the water management system used, whereas genotypes like Urano, Opale

and Salvo demonstrated an high tendency to bioconcentrate the elements in study.

2.2. Chemometric interpretation of data

The PCA analysis of data obtained allowed us to better visualize the correlations among scores (rice genotypes) and loadings (the elements measured). Below are reported the score plot (Fig. 4) and the loadings plot (Fig. 5) 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, respectively.

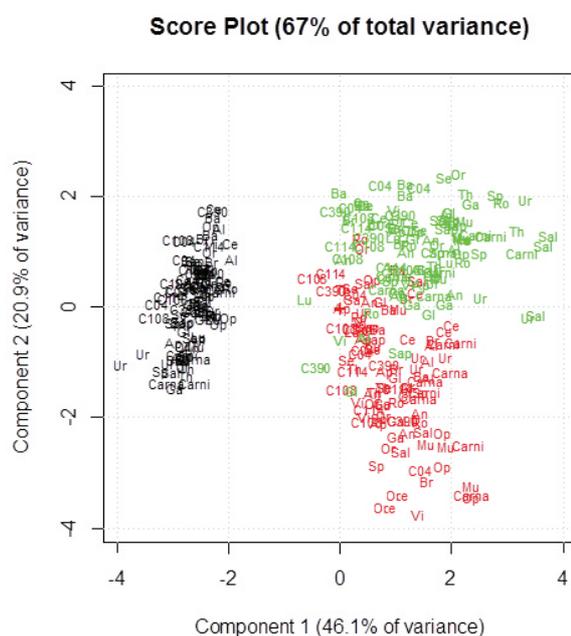


Fig. 4. Score plot of the concentrations of As, Pb, Cd, Cu, Mn, Mo, Ni and Se measured in rice grain of different 26 genotypes irrigated by CF (green), SA (red) and SP (black).

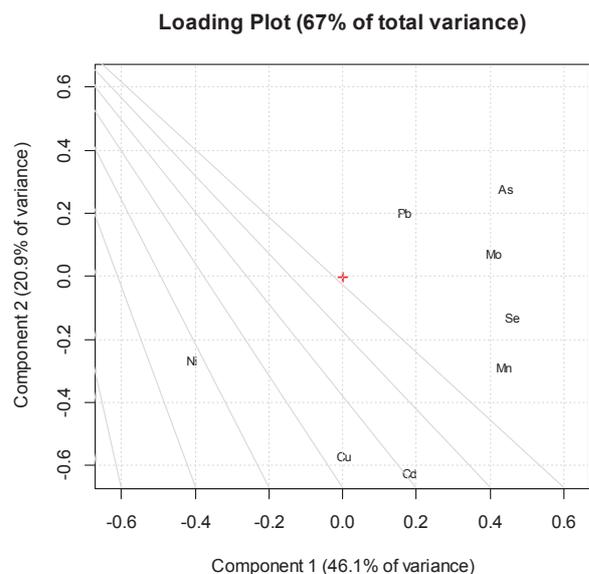


Fig. 2. Loading plot of the concentrations of As, Pb, Cd, Cu, Mn, Mo, Ni and Se measured in rice grain of different 26 genotypes irrigated by CF (green), SA (red) and SP (black).

After autoscaling, two significant components were identified giving account for 46.1% and 20.9% of the total variance, respectively. Fig. 4 substantiates that the first component discriminates SP by CF and SA rice samples, whereas the second component allows discrimination between samples irrigated by CF and SA. In addition, it is possible observe that the intrinsic dispersion of SP scores is much less than that measured for CF and SA scores. On the other hand, Fig. 5 provides strong evidence between the opposite behaviour in rice bioconcentration between Ni and the remaining elements considered, well differentiated along the PC1. Giving a closer look to Fig. 5, it is possible to note that the PC2 allow discrimination among Pb, As and Mo (positive values of PC2, elements highly bioconcentrated by CF) and Mn and Cd (negative values of PC2, elements highly bioconcentrated by SA). Furthermore, it is possible discriminate Se only along PC1, being its component in PC2 is very low, whereas the only way to discriminate Cu is along PC2, due the fact that its PC1 component is close to zero.

4. CONCLUSIONS

The concentration of eight elements of environmental concern (As, Pb, Cd, Cu, Mn, Mo, Ni and Se) has been measured by ICP-MS in rice grains from 26 genotypes cultivated in the same site and

irrigated with the same water, with the only difference in the water management system used (i.e. CF, SA, SP). Data obtained show that the bioconcentration of each element in rice grain is differently affected by the nature of each irrigation method used. In principle, we can affirm that rice irrigated by SP contains, in the average and with the sole exception of Ni – minimizes the concentration of the elements considered in comparison to values measured in rice irrigated with the other methods. Not infrequently this reduction is higher than 90% (e.g. for As, Se, Mn and Cd) and almost always is higher than 40% (Mo and Pb). A strong genotype-effect is evident for all elements in each irrigation methods tested. The score plot of PCA analysis allow to discriminate along PC1 samples irrigated by SP from those irrigated by CF and SA, whereas the loading plot is able to well differentiate between the features of bioconcentration in rice grain for each of the eight elements considered.

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