A profile of heavy metals in rice (*Oryza sativa* ssp. *indica*) landraces

Uptake of metals in rice has been reported in the context of human health and nutrition[1–4]. In particular, low levels of heavy metals like Fe, Zn, Cu and Mn in rice and other cereals are considered to be desirable for good preventive nutrition. According to FAO and WHO[1], the daily recommended intake of Fe, Zn, Cu, and Mn for an average adult is 15, 12, 1.5–3.0 mg and 2–5 mg respectively. Zn deficiency in diets entails growth retardation, immune dysfunctions and cognitive impairment[2]. Iron deficiency limits oxygen delivery to cells, resulting in fatigue and decreased immunity[3]. Deficiency of Cu causes low immune competence, while Mn deficiency may cause hardening of veins[4,5]. While Fe, Cu, Zn and Mn in cereals have nutritional value[6], certain other heavy metals (e.g. arsenic and mercury) in cereals are highly toxic, and pose serious health hazards, including cancer and disruption of the central nervous system[7,8].

Several traditional rice varieties are considered in folk medicine to have high nutritive and therapeutic value. Some of these land races are known in indigenous cultures to cure anaemia in women during and after pregnancy[9], and many of them (e.g. Kalabhat, Navara, Norungan, etc.) are now known to contain high levels of iron[6]. Thousands of folk rice landraces are yet to be screened for their micronutrients content and therapeutic potential. Examination of heavy metal content in different varieties of rice is therefore necessary to understand the dynamics of nutrient uptake in rice from different types of soil, containing heavy metals of known beneficial or deleterious effects on human health. Profiling of metals in different rice landraces would also uncover the poorly known nutritive and/or therapeutic properties of different rice landraces. This study is a contribution to generate a database of metal content of a large number of landraces of *Oryza sativa* ssp. *indica*, accessed from different regions of India and Bangladesh.

From the accession of 1030 folk rice landraces, grown every year on Basudha farm located in Rayagada district of Odisha (www.cintdis.org/basudha), we selected 130 rice landraces for profiling of heavy metals. Each rice sample was collected from the core samples of panicles, harvested in 2014 and 2015, and analysed in the Department of Chemistry, Indian Institute of Technology-Madras, Chennai.

Ten samples of each landrace were tested. Each sample, weighing 500 mg, was dissolved in 5 ml HNO_3, 2 ml H_2O_2 and 1 ml water, and digested in an Anton Paar Multiwave 3000™ microwave digester for 15 min at 800 W. After cooling, the solution was further diluted 10 times for analysis of different metal ions using an inductively coupled plasma mass spectrometer (ICPMS). After digestion, the solution was examined for silver ion concentration, using Perkin Elmer ICPMS (model NexION 300X™). XPS

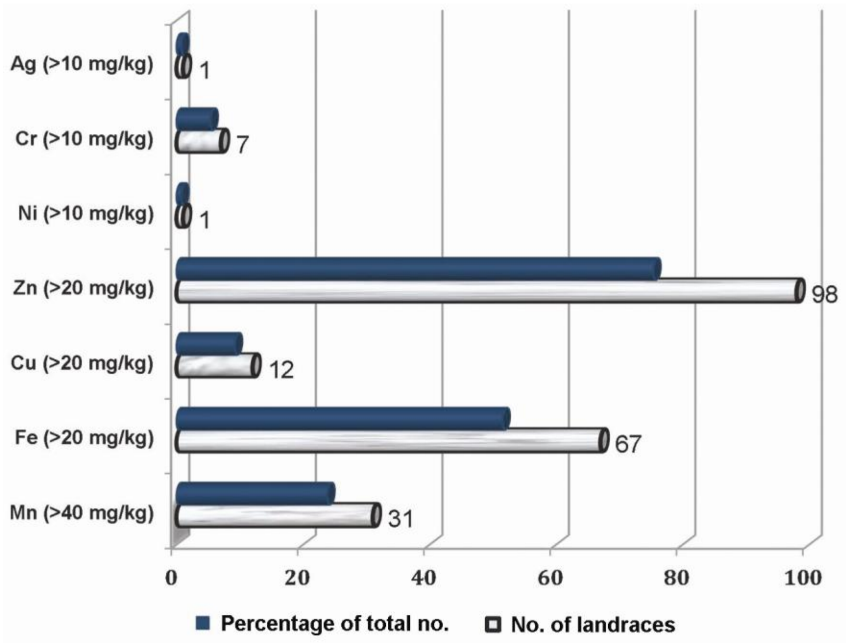

Figure 1. Number of proportion of rice landraces with high levels of metal concentration in the grains.

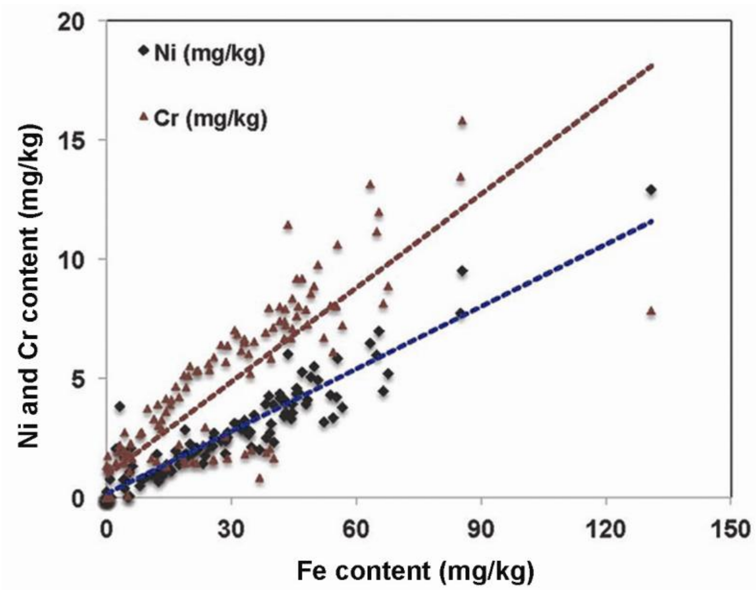

Figure 2. Regression of Ni and Cr concentration against Fe concentration in decorticated grains of 130 rice landraces.

Table 1. High concentrations of heavy metals (mg/kg) in decorticated grains of selected rice landraces

Landrace*	State of origin	Cr	Mn	Fe	Ni	Cu	Zn	Ag
Aurar	Assam	1.63	51.01	40.1	2.3	4.51	37.39	0.41
Bhuri Shulah	West Bengal	**15.8**	38.5	85.5	9.5	3.4	33.1	2.5
Cheena Kamini	West Bengal	8.9	48.7	67.6	5.2	2.2	20.3	0.3
Deulabhog	West Bengal	7.2	34.0	56.6	3.8	2.5	27.3	0.1
Dudhe bolta	West Bengal	7.8	26.0	**130.9**	**12.9**	0.7	24.8	0.0
Garib-sal	West Bengal	1.51	35.02	11.99	1.79	19.28	**155.31**	**15.61**
Gentu	West Bengal	0.0	127.9	0.0	0.0	28.4	18.3	0.2
Kabiraj-sal	West Bengal	6.8	28.3	31.4	3.0	0.4	20.3	0.0
Kalo Bhat	Odisha	1.61	53.13	6.09	1.3	3.12	40.03	0.1
Kelas	West Bengal	5.2	44.9	34.6	2.7	2.9	44.9	0.0
Keleh	West Bengal	7.4	39.4	41.6	4.1	1.7	38.4	0.1
Kermal	West Bengal	1.96	55.28	4.2	1.69	7.33	42.64	0.17
Kola Bora	Meghalaya	0.0	**138.0**	0.0	0.0	25.8	17.9	0.1
Lakkhan-sal	Jharkhand	0.0	135.1	0.0	0.0	30.8	28.8	0.3
Kurai	Odisha	6.1	27.9	54.4	3.3	1.8	23.0	0.0
Lal Getu	West Bengal	13.5	30.1	85.0	7.7	2.3	22.3	0.0
Leda-sal	West Bengal	12.0	24.9	65.4	7.0	1.8	28.3	5.3
Melhitte	Nagaland	1.43	46.61	2.17	2.02	2.9	31.62	0.07
Parmai-sal	West Bengal	0.0	124.6	0.0	0.0	30.7	20.7	0.1

*Landraces of known therapeutic value in folk medicine are shown in italics. Figures in bold indicate the highest value of the respective metal concentration.

measurements were made using Omicron ESCA Probe spectrometer with monochromatic Mg Kα X-rays ($h\nu$ = 1253.6 eV). Most of the spectra were deconvoluted to their component peaks using the software CasaXPS.

The results of our analysis of 10 heavy metal contents in 130 rice landraces (Appendix 1; see Supplementary material online) show that a considerable number of rice landraces are capable of taking up micronutrients like Fe, Cu, Zn and Mn. Among these, Kalo bora, Kelas, Garib-sal, Parmaisal and Kabiraj-sal are known in folk medicine to have therapeutic properties[10]. Many of these landraces contain up to 13-fold greater concentration of Fe and Zn than transgenic metal-fortified rice. For instance, the transgenic high-iron rice IR68144-2B-2–2-3, developed at the International Rice Research Institute, the Philippines is reported to contain 9.8 mg/kg iron, used in feeding experiments[11]. On comparison, at least 67 folk landraces from our sample contain >20 mg/kg iron (Figure 1). Clearly, these folk rice varieties have enormously high potential of remedying dietary iron and zinc deficiencies in the country, without the need to invest in developing transgenic iron-fortified rice.

Ni and Cr contents of rice grains are significantly high in most of the landraces that also have high Fe levels. We find a strong relationship of Ni and Cr with Fe concentration in the 130 landraces under study (Figure 2). The slopes of both regressions ($R^2 = 0.71$ for Cr, $R^2 = 0.88$ for Ni; $P < 0.01$) indicate a common metabolic pathway likely involved in the uptake of Fe, Ni and Cr together in rice rhizosphere. It is known that the NADPH-dependent ferric chelate reductase (AtFRO2) reduces Fe^{3+} to Fe^{2+} in the cereal root cell membrane[12]. Upon reduction, Fe^{2+} is transported into the root epidermal cells by the divalent metal transporter IRT1, which also transports Zn, Mn and, Cd, Co and Ni[13,14]. However, to explore in detail the exact metabolic pathways for the uptake of these metals is beyond the scope of this study.

Our study records several landraces with high Zn, Cu and Mn contents in the grains. The decorticated grains of one of these landraces, Garib-sal, show an extraordinarily high concentration (155.3 mg/kg) of Zn. Especially high levels of Mn are recorded in Reda dhan (130.7 mg/kg), Lakkhan-sal (135 mg/kg) and Kola bora (138 mg/kg). Considerably high (>20 mg/kg) levels of Cu were detected in the grains of Lata-sal, Lakkhan-sal and Parmai-sal. Figure 1 shows the frequency of landraces (among the 130 landraces under study) with high levels of Mn (>40 mg/kg), Fe, Cu and Zn (>20 mg/kg), as well as Ni, Cr and Ag (>10 mg/kg).

This study identifies a few landraces which are capable of bioaccumulating silver – an unusual property, hitherto unknown in rice biology. Silver concentration in agricultural crops is reported to not exceed 0.1 ppm, and is yet unknown to bioaccumulate in cereals[15,16]. In view of this, Garib-sal appears to be a unique landrace, capable of assimilating up to 15 mg/kg of silver from the soil (see Appendix 1 (see Supplementary material online) and Table 1). This landrace was originally accessed from the district of Birbhum, West Bengal, and is currently grown only on Basudha farm[7].

In traditional folk medicine of Bengal, Garib-sal was once recommended as a diet for patients with gastro-intestinal infections (D.D., unpublished). The presence of silver in this rice might likely have a therapeutic effect by killing pathogenic microbes in the human gut. Prophylactic screenings are warranted to confirm the possible curative efficacy of this rice on gastrointestinal diseases.

Thus, the present study reported the differential levels of accumulation of 12 heavy metals, including Cu, Fe, Mn, Zn and Ag, in decorticated grains of 130 rice landraces. The study also reported the extraordinary uptake and bioaccumulation of metallic silver by a rare rice landrace, Garib-sal. Most of the landraces examined here, including Garib-sal, have disappeared from rice farms[7,17] as a result of farmers' preference for improved varieties[18]. The extinction of these and other landraces from rice farms indicates the extent of loss of a wealth of indigenous rice genetic diversity, and their nutritive and therapeutic services in the wake of modernization of agriculture.

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Eminence of heavy metal accumulation in fishes and crustaceans from the Gulf of Khambhat, India

Heavy metals are found naturally in micro quantities in all aquatic systems. In fact, some of them are essential micronutrients for living organisms. However, they became highly toxic to the organisms when present in higher concentrations[1,2]. These metal concentrations have been altered in the ecosystem by indiscriminate anthropogenic activities and dispersed into the water as well as sediment column[3]. The metal contaminants in aquatic systems usually remain either in soluble or suspension form and are taken up by the organisms living in them. The progressive and irreversible accumulation of these metals in various organs of marine organisms leads to metal-related diseases in the long run because of their toxicity, thereby endangering the aquatic biota[4–7]. Bioaccumulation of heavy metals in marine organisms leads to the bio-magnification process, which is a serious threat to the ecosystem and risk to its consumers. Various species of fish are used as bio-indicators of metal pollution[8]. The concentration of heavy metals in aquatic organisms can clearly depict the past as well as the current pollution status of the environment in which the organisms live[9].

The Gulf of Khambhat has its geographic proximity to two industrially progressive states of Gujarat and Maharashtra and hence acquires significant importance from the pollution point of view. It provides transportation avenues and effluent disposal sink for the industries in these two states. The unique geomorphology of the Gulf of Khambhat is its wide open around 230 km at southern side, reducing towards northeast reaching 5 km wide between Sabarmathi and Mahi river mouths (Figure 1). Its total length is about 250 km (ref. 10). Currents in the Gulf are mainly tidal and monsoonal in origin, dominated by barotropic tides[11]. Further, Gujarat coast has the highest tidal amplitude along the Indian coast and is located nearer to the Tropic of Cancer. This highly dynamic environment also includes oil exploration along the Tapi River basin (Figure 1). Hence a study was carried out to understand the metal profile in the body tissues of marine biota around the oil rigs area. The vibrant nature of the Gulf of Khambhat keeps these contaminants usually in suspension form as the bottom sediments are dispersed into the water column during major part of the year. This leads to high suspended matter load in the Gulf water than would occur in the open marine systems[12]. As a result, seafood, particularly fishes and crustaceans which have great local consumption and export value, is considerably affected[9]. In the present correspondence, we report the status of metal accumulation in some marine biota like fishes (*Harpodon*