Environmental and Health Implications of the Correlation Between Arsenic and Zinc Levels in Rice from an Arsenic-Rich Zone in Cambodia

Tom Murphy,1 Kim Irvine,2 Kongkea Phan1, David Lean,3 Ken Wilson4

1 International University, Phnom Penh, Cambodia
2 Nanyang Technological University, Singapore
3 Lean Environmental, Apsley, Ontario, Canada
4 Texas State University, San Marcos, Texas, USA

Corresponding author:
Tom Murphy
Tel. 855-17902023
tompatmurphy@gmail.com

Introduction

In 2016, the World Health Organization and the Cambodian Ministry of Health concluded that “The health of the (Cambodian) population has improved significantly... However, challenges remain including high maternal, child and neonatal mortality that continues to occur despite recent progress; malnutrition, especially in children and women; limited access to safe water and sanitation; and a growing epidemic of noncommunicable diseases and communicable diseases.” Decades ago, more than 600,000 wells were dug in Cambodia to reduce diarrhea, cholera, and other diseases initiated by drinking water from surface sources. Unfortunately, as in Bangladesh, wells in parts of Cambodia are contaminated by naturally occurring arsenic. In 1999, high concentrations of arsenic were found in Cambodian groundwater, and by 2006, the first cases of arsenicosis in Cambodia were reported. Drinking water had been the major source of arsenic. All rice passed the current Codex standards for arsenic. However, the bioaccumulation of arsenic into rice via irrigation with groundwater could increase the probability of cancer. In areas of Bangladesh with clean drinking water, consumption of rice with more than 200 µg/kg of arsenic is associated with significantly higher levels of cancer, and some rice in Cambodia has more than double this threshold.

Background. In parts of Cambodia, irrigation of rice with groundwater results in arsenic accumulation in soils and rice, leading to health concerns associated with rice consumption. In Bangladesh and China, low zinc levels in rice have been found in regions where arsenic levels in rice are high. Furthermore, there have been claims that zinc deficiency is responsible for stunting of children in Cambodia. There are limited data on zinc in Cambodian rice, but in rural Asia, rice is the major source of zinc.

Objectives. To provide a preliminary evaluation of the zinc content in rice grain in Preak Russey, an area with elevated levels of arsenic. The importance of zinc in rice for infants was also assessed.

Methods. Rice cultivation was evaluated in sixty farms along the Mekong River in Cambodia. Analyses for metals, total arsenic, and arsenic species in the water and rice were conducted at the University of Ottawa, Canada by inductively coupled plasma – mass spectrometry. Analysis of total zinc and arsenic in soils were analyzed in Phnom Penh using X-ray fluorescence spectrometry (XRF). Total zinc in rice was also measured by XRF analysis.

Results. Rice in the Preak Russey area contained zinc with ½ to ¼ of the 1987 Codex standard for rice in Infant Formula. Moreover, our average zinc concentration in rice samples was less than a third that recommended for zinc fortification in rice by the United Nations World Food Programme. There was a significant (α=0.05) negative correlation between the arsenic and zinc content of rice with the lowest zinc levels occurring near the irrigation wells, the source of arsenic. There was a significantly higher content of zinc in rice from farms that fertilized with cow manure.

Conclusions. Handheld XRF spectrometers are useful tools for detection of zinc levels in rice. The potential for zinc deficiency in farmers in areas of Cambodia with arsenic toxicity is high.

Competing Interests. The authors declare no competing financial interests.

Keywords. arsenic, zinc, rice, XRF, irrigation, drainage, fertilization, Cambodia

Received November 6, 2018. Accepted April 1, 2019.

J Health Pollution 22: (190603) 2019
© Pure Earth
development of children, cancer resulting in limb amputation, and death. It is thought that malnutrition might enhance arsenic toxicity in Cambodia. The leading candidate for the limiting micronutrient is zinc. Therefore, the availability of zinc should be considered in arsenic toxicity.

It has been estimated that >40% of Cambodian children are at risk of zinc deficiency. Wieringa et al. proposed that zinc deficiency in Cambodia is partially responsible for anemia and stunting in children. Greffeuille et al. estimated that 32% of Cambodian children were stunted in 2014 and proposed that zinc deficiency was a major contributing factor. This analysis is complicated by genetic hemoglobin disorders such as thalassemia which are commonly responsible for anemia and may also influence stunting. Zinc is an essential trace element required for normal growth, intellectual development, immune function, and sexual reproduction. Zinc nutrition warrants further analysis in Cambodia, especially in relation to infectious diseases, diarrheal disease, diabetes, malaria, pneumonia, linear growth retardation, and arsenic toxicity, including cancer. The importance of the relationship between zinc and cancer is illustrated by zinc deficiency restricting methylation and thus detoxication of arsenic. Zinc is also an essential component of superoxide dismutases. Superoxide dismutases are important in treating reactive oxygen species, which have a major role in cancer development.

It has been estimated that one third of the population globally is deficient in zinc. More than one billion people, particularly children and pregnant women suffer from zinc deficiency related health problems in Asia. Zinc deficiency in humans reflects the fact that half of the world’s soils are deficient in zinc. Zinc deficiency in soil results in decreased zinc content in crops, reduced productivity and enhanced plant disease. The cause of zinc deficiency is well understood. However, the management of zinc in rice cultivation is still evolving and does not exist in Cambodia and many other developing countries. In rural Cambodia children get most of their energy from rice which is inadequately supplemented with fish or meat. Fortified infant formula for children is only readily available in the large urban centers and there is no national fortification of rice with micronutrients. The presence of low levels of zinc in rice in areas rich in arsenic in Bangladesh and China has been previously detected, but unfortunately the concept is not yet widely recognized.

The cultivation of rice has been optimized for centuries by empirical methods. Soil extraction and bioassay are commonly used tools requiring considerable evaluation time. There are no simple biogeochemical programs to optimize rice cultivation. Concentrations of total elements in the bulk phase of soils cannot be used to calculate bioavailable nutrients. Geochemists can measure dissolved ions, but this is expensive and technically difficult, especially in the developing world. The surface of rice roots (the rhizosphere) has a unique biogeochemical microzone. Rice plants extrude oxygen which increases the redox of this microenvironment. The transfer of oxygen from the roots to the soil varies between rice strains and likely reflects the microbial composition of the root surface. Moreover, the microbiology of the rhizosphere is not well understood. It is thought that most of the initial steps of arsenic detoxification (methylation) are mediated by microbes in the rhizosphere. Similarly, the genetics of zinc bioaccumulation vary greatly, influences how rice varieties need to be fertilized and managed, and likely also involves microbial genetics in the rhizosphere. Moreover, many rice varieties have been developed to optimize production in different soils and climates.

Genetic manipulation and plant breeding can produce rice plants with enhanced ability for zinc assimilation. Zinc enrichment is complicated, as optimal enhancement varies by rice variety, but has been successfully implemented in India to both enhance rice production and the zinc content of rice grain. Joy et al. reviewed the effective augmentation of soils in Pakistan with zinc. Soils in Pakistan have the same general derivation as in Cambodia, the runoff from the Himalayan Mountains. Attempts to enhance zinc availability by coating rice seeds with zinc were only effective in some treatments. Moreover, reviews of the effectiveness of zinc fertilization are inconsistent and fertilization is at times insufficient to alleviate zinc deficiency. The exceptions suggest that there are important regional differences in soils, rice management, and geochemistry. Pilot-scale evaluations of zinc augmentation should be implemented prior to full-scale introduction of zinc fertilizers in Cambodia.

The objective of this study is to provide a preliminary evaluation of the zinc
content in rice grain in Preak Russey, an area with elevated levels of arsenic. We also compare the zinc content of rice grain to the Codex standards for zinc in Infant and Follow-Up Formula and to zinc levels in countries with national programs for micronutrient fortification of rice. In part, it is an evaluation of a phenomenon of chlorotic rice observed in an earlier study in Preak Russey and is an extension of that study.4 The current study took advantage of the speed and simplicity of handheld X-ray fluorescence (XRF) analysis to monitor zinc concentrations in rice grain.36,37 The rice had already been collected in the earlier study.4,38

Methods

Figure 1 shows the study area. The arsenic content of groundwater varies from extreme highs in Preak Russey on the Bassac River to low levels of arsenic in groundwater near the Mekong River in a control site called Kandal. Both sites are in many ways very similar. The Bassac River is a distributary of the Mekong River. Both sites are mainly flood plains with farms with very similar agricultural techniques. Many of the rice samples in the present analysis were collected from farmers in 2016 as part of an earlier International Development Research Center (IDRC/CRDI)-funded project.4,38 Farmers in these areas hire combines to harvest their rice. The combines start at the outside of the field and make long loops around the edge, concentrically working towards the inside of the field. This provides partial integration of the rice. Single samples from farms Preak Russey-2 and Preak Russey-9 were collected by hand and the distances from the irrigation wells were recorded. For Preak Russey-1, Preak Russey-5 and Kandal-9, nine samples per farm were collected in a grid, equal distance apart. The Preak Russey-1 farm was the only one with chlorotic rice. The rice in Preak Russey-5 was chosen as...
a control to Preak Russey-1 because the rice looked healthy (greener, taller, more productive) (Figure 2) and both fields had a similar history of limited irrigation with groundwater (less than three years). The Preak Russey-1 and Preak Russey-5 fields were 100 m apart, grew the same rice variety (IR 85), and had similar alluvial clay soils and arsenic level in their irrigation water (~1000 µg/L arsenic).\textsuperscript{3,18}

The present study mainly sampled brown rice, i.e. dehusked but not yet polished. Brown rice can be stored much longer, so it is the most common rice consumed by farmers. Rice was air dried and dehusked by hand. For the XRF analysis, rice was ground with a generic Chinese food processor (Electrical Powder Grinder DB-200 g). Between samples, tools were wiped first with a wet cloth and then with a dry cloth. Attempts to grind rice with a mortar and pestle were ineffective in producing a fine powder. The rice was ground for less than 30 seconds to avoid overheating the rice. For samples larger than 100 ml, this method worked well, but smaller samples required long delays to avoid heating the sample. We chose not to add water for grinding, but this option should be considered in the future. Adding water reduces the chance of an explosion, but would require the samples to be re-dried after grinding. Grinding freshly collected rice might be considered prior to drying. There are commercial grinders with either a centrifugal action to minimize grinding time and heating or that use liquid nitrogen to assist in grinding.\textsuperscript{39,40} The risk of a major explosion with small samples is modest, but from 1970 to 2010 there were 600 explosions in grain processing facilities in the USA with 250 fatalities and >1000 injuries.\textsuperscript{41}

We used two different Niton XL3t GOLDD handheld XRF analyzers and a Bruker S1-600 Titan XRF analyzer for our analysis. Different XRF units were used in the present study due to availability issues. We used a two-minute analysis time with Soil Mode. All samples were processed using the sample cup method recommended by Thermo Fisher Scientific with Mylar film (Figure 2 in Murphy et al.).\textsuperscript{42} For rice, the following certified reference materials (CRMs) were used: 180-600 (soil), NIST 1568b (rice flour), CRM NIST 2710 (soil), and silica for a blank for quality assurance/quality control purposes (Table 1).\textsuperscript{4,38,43} The replication for all samples was very good, with a coefficient of variation of 6.87±7.47% for 44 samples. For four rice samples, samples were ground to a fine powder with the food processor and the results were compared to the XRF analysis of the whole grain (Table 2). The analysis of whole grains of rice was very consistent (average coefficient
of variation 4.48±2.28%). However, analysis of the unground rice produced much higher zinc concentrations in all but one sample. The rice bran is partly a surface layer on rice grain and since the handheld XRF analyzer has weak x-rays, only the first few surface millimeters of the grain were analyzed. X-ray fluorescence analyzers have been used to measure the elemental composition of unground rice, but inductively coupled plasma optical emission spectrometry (ICP-OES) analysis was used to standardize the XRF analysis. An XRF apparatus was not available for a sufficient period of time to evaluate the variability of analysis of samples ground only with a mortar and pestle. The rice might not have needed to be ground to a fine powder. For soils, the measured mean and standard deviation of the XRF analysis of zinc in four soil CRMs (180-600, 180-646, 180-649, 180-661) were 9±12% of the certified values. The means were within 3% of the certified values for the two CRMs closest in concentration to the samples (Table 1 in Murphy et al.).

Arsenic speciation of rice was performed at the University of Ottawa by inductively coupled plasma – mass spectrometry according to the United States Environmental Protection Agency (USEPA) Method 200.8. Arsenic species including arsenic(III), arsenic(V), monomethylarsonic acid and dimethylarsinic acid were quantified using the method developed by Agilent Technologies. Details can be found in earlier publications. An interview form was developed using preliminary visits to farms in the study areas in 2014 and 2015. The form was designed to quantify aspects of the farming methods and to facilitate communications and collaboration with the farmers. Details of these surveys conducted in an earlier study are reported in the Supplement to Appendix 2 of the IDRC/CRDI report and Murphy et al.

Statistical analyses used Excel and VassarStats.

Results

There was a significant relationship between the zinc and total arsenic content of the brown rice grain (Figures 3 and 4). All data were used in Figure 4. In Figure 3, three samples were excluded that were purposefully collected to reflect either close proximity to the irrigation well (Preak Russey-2 farm, near well) or furthest distance from the irrigation wells (Preak Russey-2 farm, far from well).
well and Preak Russey-9 farm, far from well). In either case, (Figures 3 and 4) the $R^2$ values were significant, but better when only the integrated samples (mean of the fixed grid of 9 replicates or collected by combines) were included in the analysis. The total zinc content of soils was not significantly correlated to the zinc content of the rice (data not shown). The zinc and arsenic results for site Preak Russey-1 and site Preak Russey-5 best illustrate this relationship. In both of these fields, rice and soil was sampled at 9 sites located within a fixed grid. The mean total zinc content of soil from site Preak Russey-1 and site Preak Russey-5 was 93.8±7.6 and 92.8±8.8 µg/g, respectively. By comparison, the mean total zinc of brown rice for Preak Russey-1 and Preak Russey-5 was 10.1±0.4 and 23.2±0.6 µg/g respectively (Table 3). Although differences in the zinc content of the rice grain were significant ($t$ test; $α=0.05$), there was no significant difference in total zinc content of the soil. The rice sample with the least zinc (5.7 µg/g) came from near the irrigation well of site Preak Russey-2 where the soil had 95 µg/g of arsenic, which is about twice the arsenic level of the Dutch remediation guideline requiring consideration of intervention or remediation (55 mg/kg).

There are other very important differences in the rice from Preak Russey-1 and Preak Russey-5. Table 4 shows that the arsenite concentration of the rice from Preak Russey-1 was more than five times higher than equivalent rice in Preak Russey-5. The total arsenic of rice was higher in Preak Russey-1. Arsenite in rice from Preak Russey-1 was 34.6% of the inorganic arsenic, whereas in Preak Russey-5, arsenite was only 10.6% of the inorganic arsenic (Table 2, Figures 5 and 6).

It was observed that the rice plants of Preak Russey-1 were very different from those at the rest of the sample sites at one month prior to harvest. The leaves at Preak Russey-1 were chlorotic. The contrast to the healthy normal color of the leaves from Preak Russey-5 can be seen in Figure 2. Preak Russey-1 was the only farm with consistently chlorotic rice.

The difference in the mean zinc content of the soils between the two main sampling areas, Kandal and Preak Russey, was modest, but significant (Mann-Whitney U test, $α=0.05$), Kandal 78±12 µg/g, n=75 vs. Preak Russey 85±9 µg/g, n=26. As shown in both Figures 3 and 4, there were significant differences in zinc levels in rice whether or not the farmers raised cows (Mann-Whitney U test, $α=0.05$). The more intensive sampling of Preak Russey-5 (with cows) and Kandal-9 (no cows) supports this interpretation. Both of these farms were sampled with nine samples collected in a grid equal distance apart. Although cows are fed rice bran which is enriched in zinc (Table 5), the organic content of manure appears to be more important as a regulator of the zinc content of rice.

The mean content of zinc in groundwater irrigation water of Preak Russey farms was 3.9±4.0 µg/L. In Preak Russey, the average amount of applied irrigation water was 11,600 cubic meters per hectare. The calculated loading of zinc from irrigation is about 1% of the zinc fertilization rate suggested by the Rice Institute of 10−25 kg zinc sulfate water per ha.

This concentration of zinc was close to the detection level, but the influx of zinc from irrigation with groundwater to rice was inadequate to sustain rice growth. At times the groundwater smelled like sulfides, which would precipitate zinc in the aquifer. Similarly, from the XRF analysis of eleven

| Site    | Mean $\text{As}^{3+}$ | $\% \text{As}^{3+}/(\text{As}^{3+}+\text{As}^{5+})$ | Total arsenic | N |
|---------|-----------------------|-----------------------------------------------|--------------|---|
| PR-1    | 80.3±23.2             | 34.6±13                                        | 355.8±66.8   | 9 |
| PR-5    | 14.4±18               | 10.6±12.4                                      | 158.5±30.3   | 9 |

Abbreviations: $\text{As}^{3+}$, arsenite; $\text{As}^{5+}$, arsenate; PR1, Preak Russey-1 farm; PR5, Preak Russey-5 farm; N, number of replicates. All values are presented as µg/g.

|                  | Total zinc | N |
|------------------|------------|---|
| Brown rice       | 19.4±4.8   | 24|
| Polished rice    | 14.2±4.4   | 5 |
| Rice bran        | 34.9±1.6   | 3 |

Abbreviation: N, number of replicates. All values are presented as µg/g.
inorganic fertilizers and interviews with farmers, the loading of zinc in Preak Russey was estimated to be 0.15 kg/ha as hydrated zinc sulfate, which is less than 2% of the suggested fertilization rate of the Rice Institute.

The zinc content of Preak Russey-4 rice was the 3rd highest observed in the present study (Figure 3 and 4). This farm used a treatment ditch that removed 99% of the arsenic and 92% of the iron prior to irrigation of the field. A 94% removal of arsenic and 99% removal of iron was also observed in a treatment ditch in Preak Russey-10.

Discussion

In Asia, rice is the principal nutritional source of dietary zinc. This reflects the fact that rice is the biggest dietary component and in many rural areas, and people cannot afford to eat food that is richer in zinc, such as meat and fish. As previously mentioned, rice fed to children typically is supplemented with some fish or meat, however this supplementation is inadequate. Rice is generally believed to be the main source of zinc and it relevant to compare the zinc content of rice to major food guidelines. The current Codex standards for rice in Infant Formula or Follow-Up Formula and a similar standard proposed in 2015 for Follow-Up Formula for children 12-36 months are pertinent. These standards are expressed as 1.5 mg zinc per 100 kcal, 0.5 mg zinc per 100 kcal and 0.6 mg zinc per 100 kcal, respectively. To convert these values to the same units as zinc in rice, values were adjusted using the average energy content of rice of 3.41 kcal/g. These standards are expressed as 1.5 mg zinc per 100 kcal, 0.5 mg zinc per 100 kcal and 0.6 mg zinc per 100 kcal, respectively. To convert these values to the same units as zinc in rice, values were adjusted using the average energy content of rice of 3.41 kcal/g. There are slightly different energy contents for different types of rice, especially white rice, but these calculations are intended to illustrate the health concerns for zinc in brown rice at the study sites. Revisions being considered would produce Follow-Up Formula standards for children ages 6-12 months and 12-36 months. More zinc is required in infant food, but resolution must also consider the effect of additional zinc on other micronutrient absorption.

The farm that consistently had the lowest zinc content (Preak Russey-1, mean 10 µg/g, n=9) was lower than the current Codex standard for zinc in Infant Formula (44.1 µg/g), Follow-Up Formula (14.7 µg/g Codex 1987) and some international agencies have recommended this Follow-Up Formula standard be increased to 17.6 µg/g (Table 6). Rice in this study contained zinc with ½ to ¼ of the 1987 Codex standard for rice in Infant Formula (Table 6). Three farm sites (Preak Russey-1, Preak Russey-2, Preak Russey-9) at times had rice with less zinc than the Codex standard for rice in Follow-Up Formula for young children. A higher proposed zinc standard for rice in Follow-Up Formula for young children would result in one additional farm in the arsenic contaminated zone (Preak Russey-7) and two farms in the control site (Kandal-4 and Kandal-9) failing evaluation. Farms Kandal-4 and Kandal-7 had ≤10 µg/L of arsenic in their irrigation water, so although arsenic is a major factor influencing zinc bioaccumulation, it is not the only important variable, and may only be indirectly associated with the poor zinc content of rice grain. These results suggest that zinc levels in rice from outside of the arsenic zone may at times be inadequate for the development of healthy children. This is consistent with studies which have proposed that zinc deficiency is common in Cambodia.

Table 6 provides several examples of zinc levels in rice.

For children ages 6-12 months and 12-36 months. More zinc is required in infant food, but resolution must also consider the effect of additional zinc on other micronutrient absorption.

The farm that consistently had the lowest zinc content (Preak Russey-1, mean 10 µg/g, n=9) was lower than the current Codex standard for zinc in Infant Formula (44.1 µg/g), Follow-Up Formula (14.7 µg/g Codex 1987) and some international agencies have recommended this Follow-Up Formula standard be increased to 17.6 µg/g (Table 6). Rice in this study contained zinc with ½ to ¼ of the 1987 Codex standard for rice in Infant Formula (Table 6). Three farm sites (Preak Russey-1, Preak Russey-2, Preak Russey-9) at times had rice with less zinc than the Codex standard for rice in Follow-Up Formula for young children. A higher proposed zinc standard for rice in Follow-Up Formula for young children would result in one additional farm in the arsenic contaminated zone (Preak Russey-7) and two farms in the control site (Kandal-4 and Kandal-9) failing evaluation. Farms Kandal-4 and Kandal-7 had ≤10 µg/L of arsenic in their irrigation water, so although arsenic is a major factor influencing zinc bioaccumulation, it is not the only important variable, and may only be indirectly associated with the poor zinc content of rice grain. These results suggest that zinc levels in rice from outside of the arsenic zone may at times be inadequate for the development of healthy children. This is consistent with studies which have proposed that zinc deficiency is common in Cambodia.

Table 6 provides several examples of zinc levels in rice.

In a major study of zinc in Bangladesh, Williams et al. found consistently lower levels of zinc than our limited evaluation. Bangladesh has a longer history of using groundwater irrigation than Cambodia and it often produces
more crops per year than Cambodia, both of which may enhance zinc deficiency. In 2002, Prasad stated that the severity of problems caused by zinc deficiency is not well managed or understood. Assessments of zinc deficiency are complicated and regulators need better methods of evaluating deficiency and treatment.

One of the first papers on zinc deficiency in rice stated that a rice disease in northern India was caused by zinc deficiency. Zinc deficiency reflects long periods of flooding of rice, alkaline conditions, use of high producing rice varieties, and fertilizers to boost production and multiple crops. If more irrigation water was available, a greater number of Cambodian farmers would grow a second crop and some farmers might grow a third crop. Better farm management of zinc is needed. Although improved fertilizers appear to be the most common recommendation to alleviate zinc deficiency, that is perhaps not the best solution for Preak Russey. Actual zinc demand reflects the specific field and can be higher with long periods of flooding, poor drainage, alkalinity, high arsenic or high iron, and these are all problems in Preak Russey. Reducing conditions caused by long periods of flooding inactivates zinc presumably by sulphide precipitation; thus, in reducing conditions little arsenic is required to inactivate what bioavailable zinc remains in solution.

The total zinc content of the Preak Russey soils (85±9 µg/g, n=75) and Kandal soils (78±12 µg/g, n=26) was greater than the suggested critical zinc deficiency threshold of ~10 µg/g and the suggested baseline for good zinc nutrition of soils of 60 µg/g. In addition, the zinc content of soils in the present study was similar to two areas in Bangladesh (74±17, and 97±24 zinc) where Williams et al. observed high concentrations of arsenic and low levels of zinc in rice grain. Both phosphate (an anion) and iron (a cation) can interfere with zinc bioavailability, and thus the interference is more complicated than just the ionic charge. The potential for iron interference in zinc assimilation cannot be disputed or confirmed by our study. The concentration of iron in irrigation wells in Preak Russey was 9600±6600 µg/L, n=20. Initially this iron was in solution, but it readily precipitated in the fields. The potential toxicity of iron in environments like Preak Russey has been reviewed elsewhere. However, there was no significant correlation between iron (or any other cation) in the irrigation wells and zinc in the rice grain. This sampling method may not be the most appropriate for resolving this issue. Furthermore, XRF analysis was not sensitive enough for iron in rice grain and another method of analysis should be used in future studies.

Long periods of field flooding increase soluble arsenic and iron levels, produces sulfide and precipitates zinc. However, the content of arsenic and zinc in rice grain does not only reflect the geochemistry of soils. Arsenic interferes with several metabolic pathways and
zinc is an essential part of many of these same pathways in rice. The uptake, reactivity and transportation of arsenic and zinc are enhanced/mediated by metal transporters and chelators.\textsuperscript{59-61} The production of chelators is induced both to enhance the availability of zinc and to reduce the toxicity of arsenic. The chelator nicotianamine is primarily produced to make zinc more bioavailable, but researchers stress that other chelators like phytochelatin are mainly produced to detoxify arsenic by helping sequester it in vacuoles.\textsuperscript{60} Phytochelatin can also react with zinc and any imbalance in chelation created by attempts to reduce arsenic toxicity might reduce zinc availability. Raab \textit{et al.} stated that the production of phytochelatin begins before obvious toxicity and might be a good signal of imminent suppression of productivity.\textsuperscript{62} In addition, chelation of metals can suppress production of reactive oxygen species which is an important means of arsenic toxicity.\textsuperscript{53,64} Many reactions occur in synchrony and an imbalance caused by arsenic toxicity may result in lower levels of zinc in the rice grain.

X-ray fluorescence analysis is currently the best protocol for monitoring zinc in rice grain in Cambodia. It is simple, fast, and inexpensive to operate. Handheld XRF units also are powered by rechargeable batteries. Unfortunately, the electrical supply in Cambodian laboratories is unreliable and damage to more complicated equipment is common and often cannot be repaired without external donor assistance.

As shown in Figure 4, the two major outliers Preak Russey-2 farm, far from well and Preak Russey-9 farm, far from well demonstrated a normal zinc content because their exposure to arsenic contaminated irrigation water was mediated by their greater distance from the well (65 m and 120 m). As demonstrated in an earlier publication, most of the arsenic precipitated in the paddy fields near the irrigation pumps (Figures 3 and 4 in Murphy \textit{et al.}).\textsuperscript{38} Avoiding the use of irrigation with groundwater rich in arsenic and iron water would by itself significantly enhance the concentration of zinc in rice. The treatment ditch that some farmers used to remove arsenic from irrigation water would also accomplish this objective.\textsuperscript{62} The zinc content of Preak Russey-4 rice was the third highest observed in the present study (Figure 3 and 4). This farm used a treatment ditch that removed 99% of the arsenic and 92% of the iron prior to irrigation of the field. The removal of arsenic was likely volatilization of trimethylarsine gas.\textsuperscript{62} The iron is thought to have precipitated. The removal of 94% of the arsenic and 99% of the iron was also observed in a treatment ditch in Preak Russey-10, but no rice was able to be procured from that farm. At times, it was an advantage to monitor farms, not just potted rice, but some farmers were concerned about the possible negative impact to their sales and were reluctant to cooperate. Treatment ditches are a promising technique for irrigating rice with groundwater and further analysis is needed to substantiate and optimize that treatment process.

Zinc in soils is made unavailable to rice by long durations of flooding, but drainage of rice paddies can enhance the bioavailability of zinc.\textsuperscript{65} The critical aspects of geochemistry and seepage are not yet adequately characterized to guide the frequency of drainage or the design of ditches required to enhance zinc availability. The Preak Russey-1 site had a ditch on one side of the paddy field only, whereas the farm Preak Russey-5 that produced rice with much greater zinc content had ditches on all four sides.

There was considerable seepage from the paddy field of Preak Russey-2, but not Preak Russey-1. Preak Russey-2 had the rice sample with the least zinc (5.7 µg/g), and this sample was collected near the irrigation well where the soil had the highest level of arsenic (95 µg/g). Perhaps because this farm was slightly elevated, it had sufficient drainage to improve the bioavailability of zinc in most of the field; the rice further away from the pump had more moderate levels of zinc. The apparent stagnation in Preak Russey-1 might have resulted in greater levels of arsenic in rice proportional to the arsenic in soil in all farms in the present study (Figure 2 in Murphy \textit{et al.}).\textsuperscript{38} The most relevant reflection of the importance of redox on rice in the Preak Russey-1 and Preak Russey-5 sites is the 3–4 fold higher levels of arsenic, either as a proportion of inorganic or total arsenic, respectively (Table 4, Figures 5 and 6 in this current study, and Table 2 in the IDRC/CRDI Report).\textsuperscript{38} Arsenite is more toxic than arsenate, but for the zinc geochemistry, the change in redox is the important issue.

Bhuiyan and Undan reported that the management of drainage in their study sites was mostly empirical.\textsuperscript{65} This also applies to Cambodia. For simple empirical management such as mid-season drainage in Cambodia, farmers need good irrigation and water storage facilities in order to assure an adequate water supply after they have drained their fields. In theory, wetlands within 5 km of Preak Russey could be used to store flood waters.\textsuperscript{82} Currently, farmers are unprepared to drain their fields and lose water that they may later need. Roberts and Slaton stated that in Arkansas, the only solution for severe zinc deficiency was first draining the field for two weeks, then fertilizing with zinc and nitrogen; the latter reflects the loss of nitrogen from draining the field.\textsuperscript{66} Applying zinc without the oxidation mediated by drainage is ineffective.\textsuperscript{66} Prevention is required or the cost of treatment is higher.
There are several reports of successful augmentation of soils with zinc.\textsuperscript{23,30} However, not all enrichments have been effective in both enhancing rice productivity and the zinc content of grain.\textsuperscript{34,35} Phosphorus fertilizers are known to interfere with zinc bioavailability.\textsuperscript{58} The apparent enhancement of zinc content of rice grain by cow manure illustrated in Figures 3 and 4 should be confirmed in a more controlled experiment. Furthermore, cow manure can both block arsenic bioaccumulation and enhance zinc bioavailability.\textsuperscript{67} Similarly, cow manure is known to enhance the bioavailability of refractory zinc in soils.\textsuperscript{68} This effect of organic matter is likely very dependent on the duration of flooding, drainage, and effect of organic matter on iron solubility and in turn on zinc solubility/adsorption.

The following analysis indicates that the manure enhancement of zinc in Preak Russey grain does not seem to reflect effective recycling of zinc. Most farms with cows had at least two adult cows and usually at least one younger animal. The typical feeding rate of rice bran was 2 kg/d per adult cow. The typical cow farmer would use about 5 kg/d of rice bran or 5 x 34.9 µg/g (bran zinc content) = 174.5 mg/d of zinc or 63692 mg zinc/year or converted to zinc sulphate (x 2.74) is 175 g zinc sulphate per year for one hectare, a typical farm size. This is less than 2% of the lowest recommended zinc dose.\textsuperscript{69} Unfortunately, farm productivity was not measured in the current study. The greater productivity of Preak Russey-5 relative to Preak Russey-1 is obvious in Figure 3 and future studies should evaluate the effect of enhanced zinc fertilization and any other aspect of rice management that would enhance zinc availability.

Direct fortification of rice with zinc is now commonly performed in several countries.\textsuperscript{90,70} De Pee (United Nations World Food Programme) argued that the standard for zinc in rice should be 60 µg/g, and 70 µg/g for individuals with zinc deficiency.\textsuperscript{69} Rice samples in our study had 28% of the recommended standard of 60 µg/g for augmented rice for the general public.\textsuperscript{69} Tacsan found that fortified rice in Costa Rica contains 19 µg/g of zinc.\textsuperscript{71} This variation likely reflects different targets of concern; small children and pregnant women require more micronutrients. Moreover, other micronutrients might be added; for example, all rice consumed in Costa Rica is fortified with folic acid, vitamins B1 (thiamin), B3 (niacin), B12 (cobalamin), E, selenium, and zinc. Folic acid would be especially relevant for parts of Cambodia where excessive amounts of arsenic are found, as folic acid is able to reduce the toxicity of arsenic.\textsuperscript{72}

Evaluations of zinc deficiency in farmers and the effect of zinc supplementation in individuals would be complicated. Sampson found that in some farms, family members with the same food and water developed arsenicosis, but others in the same family did not.\textsuperscript{73} The observations of keratosis and hyperpigmentation or hypopigmentation of the skin of farmers were noteworthy in that previous analysis and in the current study.\textsuperscript{7} The apparent changes in sensitivity to arsenic warrant further analysis and might reflect genetic variation. Sickle cell anemia can enhance zinc deficiency and individuals with sickle cell anemia appear to benefit from zinc supplementation.\textsuperscript{55} Zinc deficiency might enhance arsenic toxicity. Genetic hemoglobin disease might explain some observations of arsenic toxicity in Cambodia. Sickle cell anemia is not common in Cambodia, but other forms of genetic anemia are much more common.\textsuperscript{74} Beta thalassemia major does not apparently enhance zinc deficiency.\textsuperscript{76} However, there does not appear to have been any evaluation of the effect of other common forms of genetic anemia on zinc and thus indirectly on arsenic. Hemoglobin E and alpha thalassemia are common in Cambodia. Ideally, hemoglobin genetic abnormalities should be further evaluated in a study of zinc and arsenic in Preak Russey.

Augmentation of the diet in farms can be improved, but this is complicated by zinc bioavailability in plants, including rice and beans which are often suppressed by phytates. One current practice at the best managed farm site warrants replication elsewhere. First, cows are fed rice bran. The phytase in cows’ stomachs inactivates phytate and enhances zinc availability. Cow manure is then sprayed onto a fish pond that is designed to flood into the rice field. \textit{Esomus} fish species (flying Mekong barb) have about 200 mg/kg of bioavailable zinc.\textsuperscript{75} Other fish species are rich in zinc, but \textit{Esomus} species are especially zinc rich (>x2 beef) and warrant improved cultivation as they are currently popular and expensive. The bioavailability of zinc in other local foods rich in zinc such as guava leaves warrants analysis for treatment of zinc deficiency in poor rural populations.

Zinc deficiency is common globally, especially in Asia.\textsuperscript{20,22} In 2018, Wang \textit{et al.} reported that in China, almost half of the male population was at risk of zinc deficiency, reflecting the fact that poorer populations in China getting most of their zinc from grains, including rice.\textsuperscript{76} Zinc deficiency in Cambodia is not unusual, but the Cambodian focus on zinc has primarily been in children.\textsuperscript{9,10} The proposed standards for zinc in childrens food are three to nine times that for adults and reflects the greater zinc demand for growing children (\textit{Table 6}). The present study of Preak Russey demonstrates that zinc deficiency seems to be associated with arsenic contamination. Children are also more susceptible.

---

\textit{Correlation Between Arsenic and Zinc Levels in Rice in Cambodia}

\textit{Murphy et al}

\textit{Journal of Health & Pollution} Vol. 9, No. 22 — June 2019

\textit{10}
to arsenic toxicity than adults. The potential for an additive effect of arsenic toxicity and zinc deficiency is high. Moreover, treating zinc deficiency might lessen arsenic toxicity. It is therefore crucial to expand upon this evaluation of zinc deficiency in areas contaminated with arsenic.

It will require several years to improve the zinc content of Cambodian rice. While agriculture is being improved, zinc supplements could correct zinc deficiency in the Cambodian diet.

Conclusions

Handheld XRF spectrometers appear to be useful tools for detecting zinc deficiency in rice and the potential for zinc deficiency in farmers in areas of Cambodia with arsenic toxicity is high. The concentration of zinc in rice should be further evaluated in other areas of Cambodia, especially those in the arsenic-contaminated zone. Empirical approaches such as irrigation with surface water (low in arsenic), soil drainage and fertilization (especially manure) should be upgraded to improve the zinc content of rice. The geochemical factors regulating the bioavailability of zinc in soils need to be better understood to guide farm management and improve the zinc content of rice. Sampling should be done with geochemical and biological measurements with reference to the distance to the source of irrigation water. Zinc deficiency in farmers, especially those in the arsenic zone, should be evaluated, and if confirmed, treated by improved rice cultivation, zinc fortification of rice or encouraging use of zinc supplements by farmers.

Acknowledgements

The authors would like to extend their thanks to Mrs. Sharon Hau of Thermo Fisher Scientific for facilitating the loan of an X-ray fluorescence analyzer, Dr. Sothea Kim of the University of Health Science for loan of an X-ray fluorescence analyzer, Mr. Chong Sok Noung, the commune chief of Preak Russey for facilitating our interactions with farmers, and Dr. Frank Wieringa and Mrs. Robyn Devenish for providing useful information on the potential role of zinc on the stunting of growth in children in Cambodia. This project was inspired by the work of Dr. Mickey Sampson, former director of Resource Development International Cambodia, who passed away in 2009. This project was funded by in-kind and volunteer contributions.

Copyright Policy

This is an Open Access article distributed in accordance with Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0/).

References

1. Cambodia WHO country cooperation strategy 2016-2020 [Internet]. Manila, Philippines: World Health Organization, Regional Office for the Western Pacific; 2016 (cited 2019 Apr 11), 43 p. Available from: http://www.who.int/iris/handle/10665/246102
2. Shantz A, Chantrea C, Makara T, Phalla H, Daniell W, Bostick B, Abernethy A, Haven D. A study of options for safe water access in arsenic affected communities in Cambodia [Internet]. Phnom Penh, Cambodia: Resource Development International Cambodia; 2012 Feb [cited 2019 Apr 11], 68 p. Available from: http://techhumanface.org/Working/wp-content/uploads/2013/04/WSP-Final-Report.pdf
3. Sampson MI, Bostick B, Chiew H, Hagan JM, Shantz A. Arsenicosis in Cambodia: case studies and policy response. Appl Geochem [Internet]. 2008 Nov [cited 2018 Jul 23];23(11):2977-86. Available from: https://doi.org/10.1016/j.apgeochem.2008.06.022. Subscription required to view.
4. Murphy T, Phan K, Yumvhoze E, Irvine K, Wilson K, Lean D, Ty B, Poulaïn A, Laird B, Chan LH. Groundwater irrigation and arsenic speciation in rice in Cambodia. J Health Pollut [Internet]. 2018 Sep [cited 2019 Apr 11];6(19):Article 180911 [9 p.]. Available from: https://doi.org/10.5696/2156-9614-8.19.180911.
5. Phan K, Sthiannopkao S, Heng S, Phan S, Huoy L, Wong MH, Kim KW. Arsenic contamination in the food chain and its risk assessment of populations residing in the Mekong River basin of Cambodia. J Hazard Mater [Internet]. 2013 Nov 15 [cited 2018 Jul 23];262:1064-71. Available from: https://doi.org/10.1016/j.jhazmat.2012.07.005. Subscription required to view.
6. Banerjee M, Banerjee N, Bhattacharjee P, Mondal D, Lythgoe PR, Martinez M, Pan J, Polya DA, Giri AK. High arsenic in rice is associated with elevated genotoxic effects in humans. Sci Rep [Internet]. 2013 Jul 22 [cited 2018 Jul 23];3:Article 2195 [8 p.]. Available from: https://doi.org/10.1038/srep02195
7. Vibol S, Hashim JH, Sarmani S. Neurobehavioral effects of arsenic exposure among secondary school children in the Kandal Province, Cambodia. Environ Res [Internet]. 2015 Feb [cited 2019 Apr 11];137:329-37. Available from: https://doi.org/10.1016/j.envres.2014.12.001. Subscription required to view.
8. Gollapally IG, Gascoigne AC, Holmes C, Kamp EM, Jenni K, Yath SB. Arsenic and amputations in Cambodia. Asian Biomed. 2010 Jun;4(3):469-74.
9. Johnston R, Conkle J. Micronutrient deficiencies and interventions in Cambodia: information for improved programming [Internet]. Washington, D.C.: U.S. Agency for International Development; 2008 [cited 2019 Apr 11]. Available from: https://www.spring-nutrition.org/publications/projects/a2z/micronutrient-deficiencies-and-interventions-cambodia-information-improved
10. Wieringa FT, Dahl M, Chamnan C, Poiret E, Kuong K, Sophonneary P, Sinno M, Greffeuille V, Hong R, Berger J, Dijkhuizen MA, Laillon A. The high prevalence of anaemia in Cambodian children and women cannot be satisfactorily explained by nutritional deficiencies or hemoglobin disorders. Nutrients [Internet]. 2016 Jun 7 [cited 2019 Apr 11];8(6):Article 348 [12 p.]. Available from: https://doi.org/10.3390/nu8060348
11. Greffeuille V, Sophonneary P, Laillow A, Gauthier L, Hong R, Hong R, Poiret E, Dijkhuizen M, Wieringa F, Berger J. Persistent inequalities in child undernutrition in Cambodia from 2000 until today. Nutrients [Internet]. 2016 May 16 [cited 2019 Apr 11];8(5):Article 297 [18 p.]. Available from: https://doi.org/10.3390/nu8050297
12. Karakochuk CD, Whitfield KC, Barr SI, Lamers Y, Devlin AM, Vercauteren SM, Kroeun H, Talukder
19. Prasad S, Gupta SC, Tyagi AK. Reactive oxygen species (ROS) and cancer: role of antioxidative nutraceuticals. Cancer Lett. [Internet]. 2017 Feb 28 [cited 2019 Apr 11];387:95-105. Available from: https://doi.org/10.1016/j.canlet.2016.03.042 Subscription required to view.

20. Prasad AS. Zinc deficiency: has been known for 40 years but ignored by global health organizations. Br Med J. 2003 Feb 22;326(7386):409-10.

21. Alloway BJ. Soil factors associated with zinc deficiency in crops and humans. Environ Geochem Health [Internet]. 2009 Oct [cited 2019 Apr 11];31(5):537-48. Available from: https://doi.org/10.1007/s10653-009-9255-4 Subscription required to view.

22. Swamy BP, Rahman MA, Inabangan-Asilo MA, Amparado A, Manito C, Chadha-Mohanty P, Reinke R, Slama-Loedien HJ. Advances in breeding for high grain zinc in Rice. Rice [Internet]. 2016 Dec [cited 2019 Apr 11];9(1):49. Available from: https://doi.org/10.1186/s12284-016-0122-5

23. Joy EJ, Ahmad W, Zia MH, Kumssa DB, Young SD, Ander EL, Watts MJ, Stein AJ, Broadley MR. Valuing increased zinc (Zn) fertiliser-use in Pakistan. Plant Soil [Internet], 2017 Feb [cited 2019 Apr 11];411(1-2):139-50. Available from: https://doi.org/10.1007/s11104-016-2961-7

24. Cakmak I, McLaughlin MJ, White P. Zinc for better crop production and human health. Plant Soil [Internet]. 2017 Feb [cited 2019 Apr 11];411(1-2):1-4. Available from: https://doi.org/10.1007/s11104-016-3166-9

25. Welch RM. Importance of seed mineral nutrient reserves in crop growth and development. In: Bengel Z, editor. Mineral nutrition of crops: fundamental mechanisms and implications. New York, NY: Food Products Press; 1999. p. 205-26.

26. Williams PN, Islam S, Islam R, Jahiruddin M, Adomako E, Soliaman AR, Rahman GK, Lu Y, Deacon C, Zhu YG, Meharg AA. Arsenic limits trace mineral nutrition (selenium, zinc, and nickel) in Bangladesh rice grain. Environ Sci Technol [Internet], 2009 Nov 1 [cited 2019 Apr 11];43(21):8430-6. Available from: https://pubs.acs.org/doi/10.1021/es901823t Subscription required to view.

27. Duan G, Liu W, Chen X, Hu Y, Zhu Y. Association of arsenic with nutrient elements in rice plants. Metallomics. 2013 Jan;5(7):784-92.

28. Larsen M, Santser J, Oburger E, Wenzel WW, Guld RN. O2 dynamics in the rhizosphere of young rice plants (Oryza sativa L.) as studied by planar optodes. Plant Soil. 2015;390(1-2):279-292.

29. 29 Jia Y, Huang H, Zhong M, Wang FH, Zhang LM, Zhu YG. Microbial arsenic methylation in soil and rice rhizosphere. Environ Sci Technol [Internet]. 2013 Apr 2 [cited 2019 Apr 11];47(7):3141-8. Available from: https://pubs.acs.org/doi/10.1021/es303649v Subscription required to view.

30. Saha S, Chakraborty M, Padhan D, Saha B, Murmu S, Batyabyl K, Seth A, Hazra GC, Mandal B, Bell RW. Agronomic biofortification of zinc in rice: influence of cultivars and zinc application methods on grain yield and zinc bioavailability. Field Crops Res [Internet]. 2017 Aug 1 [cited 2019 Apr 11];210:52-60. Available from: https://doi.org/10.1016/j.fcr.2017.05.023 Subscription required to view.

31. Masuda H, Suzuki M, Morikawa KC, Kobayashi T, Nakanishi H, Takahashi M, Saigusa M, Mori S, Nishizawa NK. Increase in iron and zinc concentrations in rice grains via the introduction of barley genes involved in phytosiderophore synthesis. Rice [Internet]. 2008 Sep [cited 2019 Apr 11];1(1):100-8. Available from: https://doi.org/10.1007/s12284-008-9007-6

32. Barokah U, Susanto U, Swamy M, Djoar DW, Parjanto. High-zinc rice as a breakthrough for high nutritional rice breeding program [Internet]. International Conference on Climate Change: Challenges and Opportunity on Environment Degradation Researches; 2017 Oct 24-26; Surakarta, Indonesia. Bristol, United Kingdom: IOP Publishing; 2018 [cited 2019 Apr 11]. 8 p. (IOP conference series: earth and environmental science; vol. 129). Available from: http://iopscience.iop.org/article/10.1088/1755-1315/129/1/012004

33. Slaton NA, Wilson CE, Ntamatungiro S, Norman RJ, Boothe DL. Evaluation of zinc seed treatments for rice. Agron J [Internet]. 2001 Jan [cited 2019 Apr 11];93(1):152-7. Available from: https://pdfs.semanticscholar.org/d2e8/48d17271fcee41e333ad3e3ebd807f017121.pdf

34. Phattarakul N, Rekarasem B, Li L, Wu LH, Zou CQ, Ram H, Sohus VS, Kang BS, Suresh H, Kalayci M, Yazici A, Zhang FS, Cakmak I. Biofortification of rice grain with zinc through zinc fertilization in different countries. Plant Soil [Internet]. 2012 Dec [cited 2019 Apr 11];361(1-2):131-41. Available from: https://doi.org/10.1007/s11104-012-1211-x Subscription required to view.

35. Yin HJ, Gao XP, Stomph T, Li L, Zhang FS, Zou CQ. Zinc concentration in rice (Oryza sativa L.) grains and allocation in plants as affected by different zinc fertilization strategies.Commun Soil Sci Plan Anal [Internet]. 2016 [cited 2019 Apr 11];47(6):761-8.
Available from: https://doi.org/10.1080/00103624.2016.1146891. Subscription required to view.

36. Patridge NG, Palmer LJ, Millham P, Guild GE, Stangoulis JC. Energy-dispersive X-ray fluorescence analysis of zinc and iron concentration in rice and pearl millet grain. Plant Soil [Internet]. 2012 Dec [cited 2019 Apr 11];361(1-2):251-60. Available from: https://doi.org/10.1007/s11104-011-1104-4. Subscription required to view.

37. Rao DS, Babu PM, Swarnalatha P, Kota S, Bhadana VP, Varaprasad GS, Surekha K, Neeraja CN, Babu VR. Assessment of grain zinc and iron variability in rice germplasm using energy dispersive x-ray fluorescence spectrophotometer (ED - XRF). J Rice Res. 2014;7(1 & 2):45-52.

38. Murphy T, Phan K, Yunvihoo E, Irvine KN, Wilson K, Lean D, Poulian A, Laird B, Chan LH. Effects of arsenic, iron and fertilizers in soil on rice in Cambodia. J Health Polit [Internet]. 2018 Sep [cited 2019 Apr 11];38(19):Article 180910 [12 p.]. Available from: https://doi.org/10.5696/2156-9614-8.19.180910

39. Sample preparation, analysis of arsenic in rice [Internet]. Haan, Germany: Retsch; 2019 [cited 2019 Apr 11]. (About 4 screens). Available from: https://www.retsch.com/applications/knowledge-base/arsenic-in-rice/

40. Cryogenic mill with closed liquid nitrogen (LN2) auto-fill: LPM-01 [Internet]. Shanghai, China: Ruishenbao: c2013-2018 [cited 2019 Apr 11]. (About 3 screens). Available from: http://www.ruishenbao.com/en/index.php?m=content&c=index&a=show&catid=175&tid=20

41. Mittal M. Explosion hazard and safety in industries handling grain products. J Eng Res Stud. 2013 Jul-Sep;4(3)(11 p.).

42. Murphy T, Phan K, Chan L, Poulian A, Irvine KN, Lean DR, Ty B. Appendix 1 soil contamination with arsenic reflects groundwater irrigation in Kandal Province, Cambodia. In: Chan L, Murphy T, Poulian A, Laird B, Irvine KN, Lean DR, Phan K. Innovative solutions for food security/safety issues caused by arsenic contamination of rice in Cambodia. Ottawa, Canada: International Development Research Centre; 2017. 32 p. Report No: 107718-00020799-032.

43. Murphy T, Phan K, Chan L, Irvine K, Lean DR, Wilson K. Appendix 3 effect of inorganic fertilizers on arsenic contamination in rice. In: Chan L, Murphy T, Poulian A, Laird B, Irvine KN, Lean DR, Phan K. Innovative solutions for food security/safety issues caused by arsenic contamination of rice in Cambodia. Ottawa, Canada: International Development Research Centre; 2017. 32 p. Report No: 107718-00020799-032.

44. Method 200.8: determination of trace elements in waters and wastes by inductively coupled plasma-mass spectrometry [Internet]. Revision 5.4. Cincinnati, OH: Environmental Monitoring Systems Laboratory; 1994 [cited 2019 Apr 11]. 57 p. Available from: https://www.epa.gov/sites/production/files/2015-06/documents/epa-200.8.pdf

45. Agilent As speciation analysis handbook. Santa Clara, CA: Agilent Technologies; 2009.

46. Murphy T, Phan K, Chan L, Poulian A, Irvine KN, Lean DR. Appendix 2 effect of irrigation water on arsenic content of rice. In: Chan L, Murphy T, Poulian A, Laird B, Irvine KN, Lean DR, Phan K. Innovative solutions for food security/safety issues caused by arsenic contamination of rice in Cambodia. Ottawa, Canada: International Development Research Centre; 2017. 35 p. Report No: 107718-00020799-032.

47. VassarStats: statistical computation web site [Internet]. [Found in NY]: Richard Lowry; 1998 - [cited 2019 Apr 11]. Available from: http://vassarstats.net/

48. Dutch target and intervention values, 2000 (the new Dutch list) [Internet]. Amsterdam, the Netherlands: Dutch Ministry of Housing, Spatial Planning and the Environment; 2000 Feb 4. Annexes, Circular on target values and intervention values for soil remediation: [cited 2019 Apr 11]; 51 p. Available from: https://www.esdat.net/Environmental%20Standards/Dutch/annexS_I2000Dutch%20Environmental%20Standards.pdf

49. Balasubramanian V, Buresh RJ, Bell M. Zinc (Zn). [Internet]. Metro Manila, Philippines: Rice Knowledge Bank; [cited 2018 Oct 11]. (About 2 screens). Available from: http://www.riceknowledgebank.irri.org/training/fact-sheets/nutrient-management/item/zinc-fact-sheet

50. Hels O, Hassan N, Tetans I, Thisted SH. Food consumption, energy nutrition and nutrient intake and nutritional status in rural Bangladesh: changes from 1981–1982 to 1995–96. Eur J Clin Nutr [Internet]. 2003 Apr 11 [cited 2019 Apr 11];57:586-94. Available from: https://www.nature.com/articles/1601567

51. Review of the standard for follow-up formula (codex stan 156-1987). Codex Committee on Nutrition and Foods for Special Dietary Uses; 2015 Nov 27–29. Bad Soden, Germany. Rome, Italy: Food and Agriculture Organization of the United Nations; 2015 [cited 2019 Apr 11]. Available from: http://www.codexalimentarius/codexathlon/accord/2015-12-10-15-311.pdf

52. Standard for follow-up formula: CXS 156-1987 [Internet]. Rome, Italy: Food and Agriculture Organization of the United Nations; 1987 [cited 2019 Apr 11]. Available from: http://www.fao.org/fao-who-codexalimentarius/sh-proxy/en/lnk=1&url=https%3A%252F%252Fworkspace.fao.org%252Ffaostates%252Fco dex%252F Standards%252FCD%252FSTAN%252F1987%252FCXS_156.pdf

53. Suthuvtvaravut U, Abiodun PO, Chomtho S, Chongviriyapan N, Cruchet S, Davies PS, Fuchs GJ, Gopalan S, van Goudoever JB, Nel ER, Scheimann A, Spolidoro JV, Tomisirin K, Wang W, Winichagoon P, Koletzko B. Composition of follow-up formula for young children aged 12-36 months: recommendations of an international expert group coordinated by the Nutrition Association of Thailand and the Early Nutrition Academy. Ann Nutr Metab [Internet]. 2015 Oct [cited 2019 Apr 11];67(2):119-32. Available from: https://doi.org/10.1159/000438495

54. Calculation of the energy content of foods – energy conversion factors [Internet]. In: Food energy - methods of analysis and conversion factors. Rome, Italy: Food and Agriculture Organization of the United Nations; 2003 [cited 2019 Apr 11]. Chapter 3. Available from: http://www.fao.org/docrep/006/Y5022E/y5022e04.htm

55. Prasad AS. Zinc deficiency in patients with sickle cell disease. Am J Clin Nutr [Internet]. 2002 Feb [cited 2019 Apr 11];75(2):181-2. Available from: https://doi.org/10.1093/ajcn/75.2.181

56. Yoshida S, Tanaka A. Zinc deficiency of the rice plant in calcareous soils. Soil Sci Plant Nutr [Internet]. 1969 [cited 2019 Apr 11];(15)(2):75-80. Available from: http://doi.org/10.1080/00380768.1969.10432783

57. Mousavi SR. Zinc in crop production and interaction with phosphorus. Aust J Basic Appl Sci. 2011;5(9):1503-9.

58. Zinc deficiency of crops: rice (Oryza sativa L.) [Internet]. Taipei, Taiwan: Food and Fertilizer Technology Center; 2001 Sep 1 [cited 2019 Apr 11]. (About 7 screens). Available from: http://www.fftc.agnet.org/library.php?func=view&id=2011080414045

59. Yoneyama T, Ishikawa S, Fujimaki S. Route and regulation of zinc, cadmium, and iron transport in rice plants (Oryza sativa L.) during vegetative growth and grain filling: metal transporters, metal speciation, grain Cd reduction and Zn and Fe biofortification. Int J Mol Sci [Internet]. 2015 Aug 13 [cited 2019 Apr 11];16(8):19111-29. Available from: https://doi.org/10.3390/ijms160819111

60. Uriyagoda LD, Dittert K, Lambers H. Mechanism of arsenic uptake, translocation and plant resistance.
