Title: **Iodine status of soils, grain crops, and irrigation waters in Pakistan**

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ABSTRACT

A study was carried out across 86 locations of the country to investigate iodine supply potential of soils, grains and underground waters for onward design of an environmental intervention in Pakistan. Wheat crops were the principal crop in this study since it supplies 75% of calorific energy in an average Pakistani diet. TMAH-extractable iodine in soils provided a geometric mean of 0.66 µg g^{-1}, far lower than the worldwide mean of 3.0 µg g^{-1} for soil-iodine. Bio-available (water-extractable) iodine concentration had a geometric mean of 2.4% (of TMAH-extractable iodine). Median iodine concentrations in tube well sourced waters were 7.3 µg L^{-1}. Median wheat grain-iodine concentrations were 0.01 µg g^{-1}. In most of the grain samples, TMAH-extractable iodine was below detection limit of 0.01 µg g^{-1}. The highest wheat grain-iodine was measured on a soil having highest TMAH-extractable iodine. An iodine intake of 25.4 µg a day has been estimated based on median wheat grain-iodine measured and groundwater consumption compared to world health organization (WHO) recommendations of iodine intake of 150 µg a day. This nominal intake of iodine is alarming since 60% of Pakistani households don't consume iodised salt.

Keywords: iodine deficiency disorders; micronutrient; wheat flour; drinking water; iodised salt; human health

INTRODUCTION

Iodine (I) deficiency is the principle cause of preventable mental retardation and brain damage, with 1.2 billion people afflicted by iodine deficiency disorders (IDD) worldwide. Although known since 1895 about half of the world's countries continue to have some iodine deficiency. Infants, young children, pregnant and lactating women are the most vulnerable population groups because of their elevated requirements for iodine and other micronutrients. IDD causes brain damage, with irreversible mental retardation, reduced physical growth in infants and an increased risk of miscarriage or stillbirths in pregnant women (**UNICEF 2014**). Vegetarians are also particularly at risk of IDDs due to the low iodine content in fruits, vegetables and nuts (**Draper et al. 1993**). It is therefore likely that the iodine deficiency found in 37% of the Pakistani population, is a significant factor to the large mortality rate of children under five in Pakistan -- 89 per 1,000 live births (**ICCIDD 2011**).

The origins of this widespread iodine deficiency in the population of Pakistan are dietary. Cereal grains (e.g. wheat) are poor sources for many micronutrients including iodine. Since wheat provides the staple diet for the country's poorest people, they are most vulnerable to deficiency diseases. National Nutrition survey (2011) estimated that only 40% of Pakistani households consume iodized salt. A minimum intake of 150 µg iodine/day is recommended for adults to prevent IDD (**WHO 2007**). Below 100 µg day^{-1}, a series of thyroid functional and developmental abnormalities occur (**Dunn 1998**), in which symptoms can occur as goitre or result in the reduced mental and physical development of children. An iodine deficient population might suffer from an IQ reduction of 10-15% at a national scale (**Stewart et al. 2003**)

The role of iodine in endemic goitre was the first recognised association between a trace element in the environment and human health. Rocks contain little iodine and most soil iodine is derived from volatilization of methylated forms from seawater which then enters the soil–plant system via rainfall and dry deposition **(Fuge 2005; Johnson 2003b)**. Commonly known factors related to retention of iodine in soil are pH, Eh, texture, soil organic matter, Fe and Al oxides, and clay contents and their mineralogy (**Shetaya et al. 2012; Fuge 2005**). Transformation of inorganic iodine into organic forms occurs rapidly in the soil solution and the rate of loss of iodine from the soil solution is dependent upon its speciation, with iodide being lost more rapidly (minutes-hours) than iodate (hours–days) especially in high organic matter soils (**Shetaya et al. 2012)**.

Considering the calcareous nature of Pakistan's soils, it can be predicted that the iodate form of iodine might be prevalent at high pH and high carbonate contents (**Fuge 1996**; **Johnson 2003b**). Iodine concentrations of irrigation water might be best correlated with iodine concentrations of that particular geographical area (**Johnson 2003b**). Although, a coastal zone is clearly a high iodine environment that is reflected by high iodine in soil, water and crops grown thereon; no simple correlation has been observed to show any link between iodine content of soil and its distance from the sea (**Johnson 2003a**). Based on a review of 2151 citations**, Johnson (2003**) reported a worldwide concentration of 3.0 µg g^{-1} as a geometric mean for soil-iodine. In three Indian regions, the concentration of iodine in alluvial soils like that of Pakistan have been reported in the range of 3.65-9.82 µg g^{-1} (Singh et al., 2002) while in Afghanistan, soil-iodine ranged between 0.5 to 4.2 µg g^{-1} (**Watts and Mitchell 2009**).

Research in China has demonstrated that in subsistence populations consuming low-iodine foodstuffs, water can be an important dietary contributor if supplied from deep groundwater resources, which generally contain much higher concentrations of iodine than surface waters (**Fordyce et al. 2002**). The study also suggested that the iodine added in irrigation waters was only active for 1 or 2 years but it was still a very cost effective method (0.16 US$ $person^{-1}$ $year^{-1}$) to increase environmental levels. There are also many studies which raise questions on the effectiveness of salt-iodization strategy in improving human iodine levels (**Fordyce et al. 2002, 2000; Eğri et al. 2009, 2006**). **Jiang et al. (1997)** found that the irrigation method, rather than iodised salt, successfully raised the iodine status of subsistence farming-based populations in China.

Studies from the UK and Morocco have revealed that up to 10% of total iodine is water soluble (**Johnson 1980; Johnson et al. 2002**), whilst Argentina soils were reported to contain up to 42% water-soluble iodine **(Watts et al. 2010**). **Fuge and Ander (1998**) also concluded that in alkaline soils, iodate (IO_3^-) formation eliminates the chance of its re-volatilization with possible reduction in bioavailable iodine due to its fixation (**Fuge 1990**; **Fuge**

and Long 1989). **Fuge (2005**) concluded that inorganic solutes I^-, IO_3^-, and I_2 don't adsorb strongly to the mineral surfaces of layer-silicate clays. The pathway of direct absorption of iodine from the atmosphere to plants is more important than uptake of iodine from the soil through roots. The same has been confirmed using radioactive isotopes of iodine by **Asperer and Lansangan (1986)**. **Schmitz and Aumann (1994**) in a study confirmed that the water-soluble fractions of I^{127} were between 2.5 and 9.7% and for spiked I^{129} between 21.7 and 48.7%, respectively, indicating that most of the natural I^{127} was strongly bound to soil components. **Whitehead (1984**) similarly concluded that only a small proportion of the naturally occurring iodine in the soils of humid temperate regions is soluble in water, or in 0.01M $CaCl_2$, a reagent that simulates the ionic concentration of the soil solution.

Very low concentrations of iodine in wheat grains might be due to its limited translocation towards grains via phloem channels. In foliar spray studies, **Herrett et al. (1962)** concluded that I^- transport was primarily via the xylem, with little to no phloem transport, suggesting that I^- would not readily accumulate in the seed of plants. This is in agreement with studies by **Sheppard and Evenden (1992)** who reported that corn growing on a commercial soil mix containing 50 µg g^{-1} iodine had concentrations of 5.2 µg g^{-1} units iodine in the leaves and only 0.6 µg g^{-1} units in the kernels. **Muramatsu et al. (1989**) found a similar iodine partitioning relationship in field-grown rice. **Weng et al. (2009)** reported that I^{125} distribution in the young leaves of Chinese cabbage was higher than that in the old ones. **Muramatsu et al. (1995)** noted the following order (older leaves > younger leaves > grains/fruits/beans) for the concentration of iodine in plants which indicates little translocation from the leaves (**Sheppard et al. 1993**). **Johnson (2003b**) concluded that locally grown food from most areas of the world, except coastal areas, are not going to produce sufficient iodine to reach an Adults Recommended Dietary Allowance (RDA) of 150 µg day^{-1}. Iodine being non-mobile is not concentrated in the seed (**Johnson 2003b**) therefore, seed crops such as rice (and wheat) can't be considered as a good source of dietary iodine (**Fordyce et al. 2000; Tsukada et al. 2008**).

A traditional preventative solution to IDDs is to foment the consumption of iodised salt, although the strategy is effective only in cases of mild deficiencies (**Zhu et al. 2003**). About 90% of iodine in iodized salt has been reported to be wasted during production, storage, transportation, and cooking (**Chi 1993; Diosady et al. 1998**; **Zhang et al. 2002**). The **World Health Organization (2007**) suggested there may be 20% loss of iodine through processing and another 20% through cooking and food preparation practices. In an extensive study using 50 different Indian recipes, using different cooking procedures, **Goindi et al. (1995**) found the range of losses between 3 and 67%. The mean I losses ranged from 6 to 37%, though clearly the variation in results was very large in this study. Still being advocated a popular strategy, worldwide, the annual costs of salt iodization are estimated at 0.02–0.05 US$ per child covered (**Zimmermann 2008**). Despite an iodised salt campaign, Pakistan's National Nutrition Survey (NNS) in 2011 documented a reduction in iodine deficiency among school age children (6-12 years age) from 63.2% to 36.7% over the previous decade (**ICCIDD 2013**), although 2.1 million children are still born each year with mental disorders in Pakistan due to iodine deficiency in pregnant women (**APP 2013; ICCIDD 2013**). Thirty one per cent of cooking salt brands tested across the country were found negative for iodine content whereas adoption of iodised salt at a household level was only 40%, whilst goitre rate among school age children was 7%. Urinary iodine concentration (UIC) measurements below 100 μg L^{-1}, were revealed in mothers from Balochistan, AJ&K and Gilgit Baltistan provinces and in pre-school children from AJ&K and Gilgit Baltistan provinces only.

In the past, IDD has been widely studied in Pakistan as a medical issue, but this is probably the first study in Pakistan that addresses possible iodine deficiency from the viewpoint of soil, grain crops and irrigation water as dietary sources. This study will help inform effective iodine intervention programmes to plan agricultural practices that will improve the retention of iodine or increase the soil-iodine concentration for subsequent uptake by staple crops. Targeting micronutrient deficiencies such as iodine will contribute to the targeting of the Millennium Development Goals (MDG) in Pakistan; (MDG 1) reduce extreme poverty and hunger; (MDG 2, 3) reduce cognitive dysfunction and growth retardation; (MDG 4, 5) child and maternal mortality; and (MDG 6) diseases. For example, the addition of iodine to irrigation water (fertigation) in China successfully increased the concentration of iodine in spinach (**Dai et al. 2004a; 2006**). **Yuita (1982)** reported an iodine range of 0.35–1.05 μg g^{-1} in rice leaves when grown on soils containing 0.5 - 4.8 μg g^{-1} background I levels. However, in a hydroponic study (**Mackowiak and Grossl 1999**), even a treatment at 100 *μM* IO_3^- could not provide sufficient I in the rice seed to meet human dietary requirements demonstrating its limited translocation towards grains via xylem unlike the leafy vegetables.

It is well established that seafood, meat and dairy produce are quite enriched in iodine, but resource poor communities across Pakistan cannot invest in such a diversified diet, rather they have to rely on locally produced staple crops such as grain for their calorific value rather than their nutritional value. The result is a 'hidden hunger' for micronutrients. Therefore, this study was planned to: i) measure the extent and spatial distribution of iodine in soils, irrigation waters, and wheat grains across Pakistan; ii) gain a geochemical baseline understanding of the existing soil-plant transfer of I in order to focus on high risk areas; and iii) identify the onward implications for micronutrient delivery via the diet through a change in agricultural practices to fortify staple crops. The results will inform the design of larger scale follow-on studies, with dietary-health status evaluation to improve health via more effective micronutrient delivery, specifically iodine at a national scale, in particular to vulnerable groups at risk of deficiency such as pregnant mothers and children.

MATERIALS AND METHODS

Eighty-six (86) study sites were selected based on wheat growing regions all across Pakistan, stretching from Azad Jammu & Kashmir (AJ&K) highlands up to coastal areas of the Arabian Sea. All of the study sites are illustrated in Figure 1. Through this sampling strategy, almost all of the wheat growing districts of Pakistan were covered. These soils were chosen to represent a wide range in texture, physico-chemical properties, and distance from the ocean; all factors which may affect the global cycling of iodine. The range of sample types provide an opportunity to examine the correlation of total iodine content with soil properties (e.g., organic matter, clay mineralogy, soil pH, and texture) and the influence of these properties on the transport behaviour of iodine. The wheat crop was selected on the basis that 80% of the country's population consumes wheat as an essential component of daily food (**Gallup-Gilani Pakistan 2011**), supplying roughly 75% of calorific energy in the average diet (**World-Grain 2013**). Moreover, per capita consumption of wheat flour in Pakistan is 127 kg per annum, which is among the highest in the world.

Sample preparation and basic analyses

Approximately 0.2 kg of soil was collected with an augur from the top 20-cm layer of soil. Samples were gently dried in an oven at 35°C overnight to minimise the risk of I loss and then sieved using a nylon mesh to <2 mm. The soil samples were subsequently milled to <125 µm using a mortar and pestle at the Fauji Fertiliser Company (FFC) laboratories. In addition, from each farm, irrigation water samples (60 mL) were collected and filtered on-site using a 0.45-µm syringe filter. Wheat crop demonstration plots covering a 2-acre area were sown at same locations as the soil and wheat crops to evaluate the soil-plant-grain transfer of iodine. For the wheat crop, mature green flag leaves were randomly collected across 2-acre fields at each location and washed with deionised water before drying at 35°C. At harvest, representative wheat grain samples were collected from respective soil sampled fields and washed with deionised water to eliminate any contaminants/dust particles. The wheat leaves and grains were milled using a grinding mill.

The soil samples were analyzed in the FFC laboratory for $pH_{1:2.5}$, electrical conductivity ($EC_{1:2.5}$), organic matter, $NaHCO_3$-P and NH_4OAC-K according to the standard methods. For Fe, and Zn “available” concentrations, sample extracts were prepared using DTPA solution , and dilute HCl for B. Plant-available Fe, and Zn concentrations were determined by atomic absorption spectrometry technique and B by colourimetry with Azomethane-H indicator (Table 1). Using microwave assisted hydrofluoric acid digestion technique, total P, K, Fe, and Zn concentrations in the soils depicted geometric mean values of 814, 21635, 32945, and 67 µg g^{-1}.

Iodine determination

Tetra methyl ammonium hydroxide (TMAH) was reported to extract quantitatively the total iodine content from environmental samples, e.g., soils, sediments, plants, and food (**Watts and Mitchell 2009; Shetaya et al. 2012**). Alkaline extractants such as TMAH solublise humic acids (and org-I) by negative charge generation and may also cause some degree of hydrolysis of org-I compounds. In addition, TMAH releases iodate from specific sorption sites on Fe/Al hydrous oxides by replacement with hydroxide ions and negative charge generation on the oxide surface (**Yamada et al. 1996**). The methodology for the measurement of iodine in water and soil followed was based on that of **Watts and Mitchell (2009)**. For soils, 0.25 g (dry weight) of sample was weighed directly into a 15 ml poly (tetrafluoroethene) HDPE Nalgene bottle to which 5 ml of 5% TMAH was added and shaken. Sample bottles, with lids loosened, were placed in a drying oven at 70 °C for 3 h, with bottles shaken at 1.5 h. After 3 h of heating, 5 ml of deionised water was added and the bottles centrifuged at 2500 rpm for 20 min. The supernatant was removed from the top of the sample solution and diluted to a final matrix of 0.5% TMAH. Soluble iodine was determined by cold water extraction, with 12.5 ml of deionised water and 1.25 g of soil shaken for 15 min, centrifuged at 3000 rpm for 10 min and adjusted to a matrix of 0.5% TMAH for analysis. Water samples were spiked with 25% tetramethyl ammonium hydroxide (TMAH: Sigma Aldrich, Kent, UK) to result in a final solution of 0.5% TMAH. For wheat grains and leaves, the same methodology was adopted as for the soil samples (TMAH extractable I). However, the method could not produce a clean enough solution for ICP-MS analysis and most likely an incomplete extraction of iodine. Therefore, TMAH extractions were performed using a CEM MarsXpress microwave, whereby 0.25g of sample was weighed directly into the microwave vessels, 5 ml of 5% TMAH added and shaken to mix. The vessels were capped and placed in the microwave and heated at 1600W to ramp up to 70 ˚C over 10 minutes and then held at 70 ˚C for 60 minutes. This approach produced a much cleaner extract solution for the grain samples compared to the heating method used for the soil and leaf samples. After heating, the grain samples were diluted and centrifuged as for the soil and leaf samples. Certified reference materials (CRMs) were used within each extraction batch to monitor the performance of the TMAH extraction and subsequent analysis by ICP-MS; soils (GSS-2, GSS-3, GSS-5, GSS-7, GSS-8); plants (NIST 1573a tomato leaves, and GBW08503 wheat flour). All measurements were within ± 15% of target concentrations, ranging from 1 to 5 repetitions of each CRM (Table 2).

All sample solutions were analysed by a Spectro ICP-MS instrument (model ICPMS01, Spectro, UK). Samples were introduced to the ICP-MS using an Cetac ASXpress flow injection device coupled with a Cetac 500 series autosampler. During the study a combination of a Savillec C-type nebuliser with a Scott double pass spray chamber was found to be most resistant to blockages from the difficult samples. An internal standard mixture of 50 µg L^{-1} Sc, Ge, Rh, In, Te, Re and Ir in water was mixed with the sample solution via a t-piece to correct for mass and signal (Te) drift. The Spectro ICP-MS is a magnetic sector - array detector based instrument that simultaneously captures an entire mass spectra. Acquisitions were an average of 3 times 30 second integrations.

The limits of detection for the sample preparation and analysis of iodine in the current study were: waters – 0.4 ng ml^{-1}; soil – 0.05 µg g^{-1}; and vegetation – 0.02 µg g^{-1}.

Soil-to-grain transfer factors (TFgrain) for iodine were calculated as follows:

$$TF_{grain} = [IC_{grain}]^{dry} / IC_{soil}$$

where $[IC_{grain}]^{dry}$ is iodine concentration (µg g^{-1}) in wheat grains on a dry weight basis and IC_{soil} is TMAH-extractable iodine concentration (µg/g) in the corresponding soil samples. All data were subjected to analysis of correlation (ANOVA, two-way) performed using Windows based Statistix 8.1.

Background soil concentrations for organic matter, Olsen P, ammonium acetate - extractable K, DTPA-extractable Fe, Zn, and dilute-HCl extractable boron along with other physicho-chemical characteristics are given in Table 1. The soils ranged in texture from loamy sand to clay loam as per US Soil Survey classification system. Mean soil pH was recorded to be 8.1 which is characteristic of calcareous soil. Minimum pH was noted for the soils of AJ&K where due to low temperatures and high rainfall, there is leaching of basic cations compared to high pH arid areas of the region. The sampled locations were free of any salinity as mean soil salinity was noted to be 0.33 dS m^{-1}. High amount of soil organic matter were also noted for AJ&K and Mardan district of KPK province where temperate climate prevails. The soils were generally low in available phosphorus but had a satisfactory amount of potassium. Fertilization of the demonstration plots was also made on the basis of soil analysis in this study. The soils were marginally deficient in zinc and boron but had sufficient amounts of iron (> 4.5 µg g^{-1}).

RESULTS AND DISCUSSIONS

Soil Iodine

TMAH extractable iodine results in this study show that soils of Pakistan are generally deficient in iodine with an exception to some areas where high organic carbon under temperate climate prevails naturally, for example all the samples collected from AJ&K highlands, KPK and Balochistan Province where a temperate climate prevails (Figure 2 & Table 3). The TMAH-extractable soil-iodine ranged from 0.19 to 9.59 µg g^{-1}, with a geometric mean concentration of 0.66 µg g^{-1}. The mean soil-iodine concentration is significantly lower than the worldwide geometric mean of 3.0 µg g^{-1} (**Johnson 2003**) and is also lower than that of alluvium derived soils (mean 1.28 µg g^{-1}) (**Johnson 2003**). Bio-available (cold-water soluble) iodine concentrations for soil samples provided a geometric mean concentration of 2.36 % (of TMAH-extractable iodine), ranging from 0.4 and 9.0%. No significant correlation between any of the soil factors, including soil pH, soil phosphorus, DTPA-extractable micronutrients were evident with that of TMAH-extractable soil iodine, except for the soil organic matter (0.34 at P<0.001). However, if the outliers are removed then for 90% of the data there is a very weak correlation between TMAH-extractable soil iodine and soil organic matter (data not shown here).

Soil pH is the most influential factor on iodate sorption, whether retention occurs by adsorption on soil oxyhydroxides or by chemical bonding to soil organic matter; however, in soils with similar pH values, sorption is greater in soils with higher organic matter content (**Shetaya et al. 2012**). With an increase in soil pH there is a decrease in sorption of inorganic I, a behaviour that is similar to non-specific sorption of anions like Cl^-, NO_3^-, and SO_4^{2-}. Within a normal soil pH range, iodate (pKa = 0.75) and iodide (pKa = -10) are both fully dissociated where variable charge iron oxide surfaces are believed to electrostatically attract both forms. Therefore, under acidic soil conditions sorption would normally be stronger. Stronger adsorption of iodate compared with iodide has also been reported in low organic matter, acidic soils (**Fukui et al. 1996; Yoshida et al. 1992; Shimamoto et al. 2011**) which was attributed to chemical bonding of iodate to iron oxide surfaces through replacement of hydroxyl groups (**Fukui et al. 1996; Dai et al. 2004b; Um et al. 2004**).

Uptake of iodine by plants grown in soils is dependent on the availability of iodine in the soils, which is essentially governed by adsorption-desorption processes in soils. **Watts et al. (2010)** noted that mobile water extractable soil-iodine was 1–18% for La Pampa and 2–42% for San Juan province of Argentina. Coarse textured soils such as those derived from sand and alluvium are generally low in iodine (**Johnson 2003**). Higher values of iodine have been noted for temperate, high rainfall, hilly areas of Pakistan where soils are rich in organic matter (Table 3; Figure 2). Whereas arid regions of Punjab province, with minimum rainfall, demonstrated the lowest soil iodine concentrations. **Shetaya et al. (2012)** reported that iodide sorption was greater in top soils with high-organic contents than in low-organic subsoils regardless of the soil pH, suggesting that iodide sorption was much more influenced by organic matter content than pH and that iodide is mostly retained in soils by chemical incorporation in soil organic matter.

Fordyce et al. (2000) reported concentrations of soil-iodine were highest in Sri Lankan villages, although the soil clay and organic matter content appeared to inhibit the bioavailability of iodine. A highly significant, positive correlation (0.80 at $P<0.001$) was observed between TMAH-extractable soil iodine and cold-water soluble soil iodine in this study. A negative correlation (- 0.12 at $P<0.001$) was observed between TMAH-extractable soil iodine and soil pH while a correlation value of 0.13 was observed for cold-water soluble soil iodine against soil pH. In case of temperate, low pH soils of AJ&K alone, the correlation between TMAH-extractable soil iodine and soil pH was – 0.45 at $P<0.001$ whereas the correlation value was – 0.38 between the cold-water soluble iodine and soil pH for AJ&K soils group.

Organic matter plays a significant role in the retention of soil-iodine, particularly in surface soils (**Johnson 2003b**). It is probably the single most important determinant in contributing to the total iodine levels in soils as sorption of iodine in soils is directly related to the organic matter content (**Sheppard and Thibault 1992**). A high proportion of the soil's iodine (nearly 90% of the total) is organic iodine bound to fulvic and humic acids (**Hu et al. 2007**). Transformation of inorganic iodine to organic iodine plays an important role in iodine immobilization, especially in a surface soil-water system. Retention of iodine in soils may be primarily through physical association with the surfaces and entrapment in the micro-pores and structural cavities of the intricate fabric of the organic matter (**Sheppard and Thibault 1992)**. A significant, positive correlation (0.34 at $P<0.001$) was observed between TMAH-extractable soil iodine and soil organic matter for the 86 sampled locations. This correlation further improved (0.58 at $P<0.001$) when only temperate, low pH soils from AJ&K highlands were analysed, statistically. Similarly, a positive correlation (0.11 at $P<0.001$) was observed between cold-water soluble soil iodine and soil organic matter for the 86 sampled locations which got further improved (0.17 at $P<0.001$) when only temperate soils from AJ&K highlands were analysed, statistically. Thus, it would appear that it may not be appropriate to produce generic relationships across Pakistan, but only for domains of similar climate-soil type.

Irrigation water Iodine

A total of 29 water samples were collected across Pakistan from the locations where wheat crop was grown to monitor iodine uptake and its accumulation in wheat grains. The mean iodine concentration in irrigation waters was 8.5 $\mu g\ L^{-1}$, with a median value of 7.3 $\mu g\ L^{-1}$ (Table 3; Figure 3). The highest concentration for iodine in irrigation water was noted for a location at Qazi Ahmad, in the Nawab Shah District of Sindh province, approximately 200 km from the sea. The analysis of canal-fed irrigation water, collected for the comparison purpose, (sample # 29 in Online Resource 1) at one of the locations showed a significantly lower concentration of iodine (1.7 $\mu g\ L^{-1}$) compared to 8.5 $\mu g\ L^{-1}$ (mean) in underground water sampled across the country (Table 3; Figure 3). Pakistan is deficient in canal water and therefore farmers have supplemented it through the installation of more than 0.91 million tube wells to pump groundwater to irrigate their crops (**Anonymous 2010-11**). The use of groundwater may inadvertently provide an additional supply of iodine to soils and their crops, although further research is required to understand the input, residence time in soil, saturation, recharge points and transfer ratios to crops. If iodine intake is taken into account from drinking water, sourced from underground in Pakistan and consumed at 3 litres per person per day (2 litre for drinking and 1 litre for use in cooking) an iodine intake of 21.9 µg a day is estimated based on a median underground water I value of 7.3 µg L^{-1}.

Province wide, geometric mean values for irrigation water iodine were found to be 9.6, 16.2, 0.8 and 12.1 µg L^{-1} for Punjab, Sindh, KPK, and AJ&K, respectively (Figure 3). The highest mean concentration was observed for Sindh province that adjoins sea while minimum mean value is reported for KPK which is farthest from the coast and has a temperate climate. The Punjab province with an arid climate had a mean concentration of 9.6 µg L^{-1} iodine in irrigation water. A similar trend was also noted for median values of irrigation water iodine. Irrigation water samples could not be collected from Balochistan province in this study. Geometric mean and median values for irrigation waters iodine show that with increasing distance from the Arabian Sea there is a decrease in iodine contents but this needs further investigation based on large dataset. In Pakistan, canal water flows from the Himalayan Mountains towards the Arabian Sea, an opportunity that can be used for environmental iodine intervention approaches via fertigation of iodine, particularly for staple crops that require irrigation via flooding (e.g. rice) or low technology and cost agricultural practices that use flooding rather than pumping or spraying as is used for wheat grains in Pakistan. **Cao et al. (1993)** iodinated irrigation water to increase iodine in soil, crops, animals, and human beings in Xinjiang province, China. Five per cent potassium iodate solution was dripped into an irrigation canal for 12 as well as 24 days, which increased soil iodine 3-fold, and crop and animal iodine 2-fold. Median urinary iodine excretion in children increased from 18 to 49 µg L^{-1} (two groups of similar age), compared to the healthy target value of > 49 µg L^{-1}. The cost for iodinated irrigation was US $0.05 per person per year. Soil iodine remained stable over one winter, and the dripping of iodine during the second year (US $ 0.12 per person per year) resulted in a further 4-fold increase in soil iodine and a 1.8-fold increase in iodine in crops.

Plant and Grain Iodine

TMAH-extractable iodine results show that wheat grains from Pakistan are generally low in iodine from 0.01 to 0.03 µg g^{-1} in wheat flour (on dry weight basis) with a mean and median concentration of 0.013 µg g^{-1}, and 0.01 µg g^{-1}, respectively (Table 3; Figure 4), compared to a worldwide mean of 0.56 µg g^{-1}, reported by **the Chilean Iodine Educational Bureau (1952)**. In the case of flag leaf analysis, the iodine concentrations ranged from 0.12 to 0.47 µg g^{-1}, with a geometric mean of 0.22 µg g^{-1} (Table 3; Figure 5). The highest concentration of iodine in wheat grain (0.03 µg g^{-1}) was found on a soil with the highest TMAH-extractable iodine at 9.59 µg g^{-1} in the Kochlaak district of Balochistan province where a temperate climate prevails. Significant, positive correlation values of 0.55, and 0.57 were observed for TMAH-extractable soil iodine and water-soluble iodine, respectively against wheat grain iodine. Opposed to this, a weak correlation value of 0.17 was observed between TMAH-extractable iodine and wheat leaves iodine. The significant positive correlation between TMAH-extractable soil iodine and wheat grain iodine implies that an environmental intervention approach to enrich soils with iodine might be helpful in enhancing grain iodine status. For the 22 locations with grain-iodine values, correlations between TMAH-extractable soil iodine, cold-water soluble iodine, wheat leaf iodine, and wheat grain iodine, were found to be significant. Correlation between water soluble iodine and TMAH-extractable iodine for the 22 samples was highly significant, positive ($r = 0.99$, $P < 0.001$) and water soluble I ranged from 0.4–9 % of

TMAH extractable I. Correlation between wheat grain iodine and TMAH-extractable iodine was significantly positive ($r = 0.55$, $P < 0.05$). Correlation between wheat grain iodine and water soluble iodine for the 22 samples was also significantly positive ($r = 0.57$, $P < 0.05$).

Soil-to-grain transfer factors (TF) for TMAH-extractable, and water-soluble (bioavailable) iodine in this study were calculated to be in the range of 0.003 to 0.053, and 0.11 to 2.63, respectively. Soil-to-grain transfer factors (TF) for TMAH-extractable iodine encompass the values of 0.001 observed for wheat grown over podzoluvisol soil (**Kashparov et al. 2005)**. In a study by **Shinonaga et al. (2001**) the concentrations of iodine in cereal grains across 38 locations in Austria were found to be in a range of 0.0005 to 0.02 µg g^{-1}. Uptake of iodine by plants grown in soils is dependent on the availability of iodine in soils, which is essentially governed by adsorption-desorption processes in soils. The TF values correlated negatively (-0.51, and -0.45) with TMAH-extractable, and water-soluble iodine concentration of the soils in which the grain crop was sown, suggesting that soil characteristics can increase soil adsorption, and reduce plant availability, of the element (**Shinonaga et al. 2001**). Overall, TFs are low for iodine probably due to strong soil adsorption of the element in the oxic region of soils where plant root predominate (**Ashworth 2009**) but this is not the case in rice grown over flooded soils. **Dai et al. (2006)** reported that iodine concentrations in spinach plants on the basis of fresh weights increased with increasing addition of iodine. Since, the soil-to-leaf transfer factors for plants grown with iodate were about ten-fold higher than those grown with iodide therefore; iodate form can be considered as potential iodine fertiliser to increase the iodine content of leafy vegetables. In a similar hydroponic study (**Mackowiak and Grossl 1999**), the treatment at 100 µM IO_3^- could not provide sufficient iodine in the rice seed to meet human dietary requirements which make iodine fertilisation approach, at least for cereal grains questionable.

In most of the grain samples (62 of 84 locations), TMAH-extractable iodine was below the analytical detection limit therefore results of grain iodine with detectable amounts are reported here for only 22 grain sample locations across Pakistan (see Online Resource 1). The determination of iodine in food has been a challenging analytical problem for a long time (**Pennington et al. 1995**). The concentration of iodine in most foods is low. Therefore, accurate determination requires a sensitive analytical method and freedom from contamination. Per capita iodine exposure as per average wheat consumption (350 gram day^{-1}) with mean iodine concentration of 0.01 µg g^{-1} reported in this study would provide a daily iodine intake of 3.5 µg day^{-1}. Since wheat grains contribute a 75% of daily calorific value in Pakistan, the intake of iodine is severely limited from staple foods and the figure is far below the minimum recommended iodine intake of 150 µg day^{-1} (**WHO 2007**). Therefore, the diversification of dietary intake of other sources of iodine rich food like fish, milk, fruits, and iodised salt is need of the hour. For example, by consuming 5 grammes of iodised salt (having 15 µg iodine per gram of salt), an individual's additional intake of iodine might be about 75 µg a day but one has also to take into account iodine losses during process of cooking. Potential iodine deficiencies of vulnerable population groups such as pregnant women or infants, who may be exposed to low iodine levels, are not revealed by intake estimates based on average consumption data as discussed above.

CONCLUSION

Samples analysed for iodine across the country had a geometric mean soil iodine concentration of 0.66 µg g^{-1} which is significantly lower than the worldwide geometric mean of 3.0 µg g^{-1}. Median water–iodine concentrations (7.3 µg L^{-1}) were almost similar to the UK (0.40 – 15.6 µg L^{-1}) and North America (0.47 – 13.3 µg L^{-1}) (**Fuge 1989**). The highest concentrations were measured in underground waters from Sindh province (10.8 µg L^{-1}) that borders the coastal belt. However, many of the soils were consistent with iodine deficient areas reported by **Fordyce et al. (2002**) at less than 3.1 µg g^{-1}. Although the correlation of total soil-iodine with prevalence of IDD is questionable (**Stewart et al. 2003**) there is a need to understand the bioavailable fraction in order to better understand the factors that influence IDD. In most of the grain samples collected from across the country, iodine concentrations were below the detection limit (0.01 µg g^{-1}). This work has shown that high iodine concentrations have been observed on temperate soils, high in organic matter. High pH influenced iodine uptake by wheat in a negative manner whereas low pH soils of temperate regions depicted higher concentrations of iodine in grains (0.02 to 0.03 µg g^{-1} I). Soils are influential in the nutritional status of humans and animals. Geochemical mapping can stimulate investigations into the cause of diseases and aid the planning of public health corrective responses (**Abrahams 2006**). Mitigation strategies, such as the common practice of salt iodisation in Pakistan or direct supplementation are often mistaken as a conspiracy of the west (**ICCIDD 2013**). Localised mitigation strategies have been proposed for the improvement of soil-iodine, such as crop bio-fortification (**Yang et al. 2007**), addition of Chilean iodine rich nitrate fertilisers, soil improvement through addition of organic matter (**Aston and Brazier 1979; Johnson 2003b**) or iodination of well water or irrigation water (**Lim et al. 2006**). Canal water had significantly lower concentration of iodine (1.7 µg L^{-1}) compared with tube well water (8.5 µg L^{-1}). Per capita iodine intake as per average wheat consumption (350 gram day^{-1}) with a mean iodine concentration of 0.01 µg g^{-1} reported in this study would provide a daily iodine intake of 3.5 µg day^{-1}. An additional intake of 21.9 µg L^{-1} can be counted from underground water source that is used for drinking purpose almost across the country. Since wheat grain contributes a 75% of daily calorific value in Pakistan hence the total iodine intake figure of 25.4 µg a day (sourced from grains and drinking water) is far below the recommended iodine intake of 150 µg day^{-1} (**WHO 2007**) and deserves supplementation of other sources of iodine rich food like fish, milk, fruits, and iodised salt. Adoption of iodised salt at a household level is only 40% across Pakistan which suggests that 60% of the country's population is at high risk of iodine deficiency disorders. To eliminate iodine deficiency at a population scale and to ensure an equitable approach to supplementation, iodine may be either added through irrigation water that is based on gravitational flow across the Punjab and Sindh provinces. This could be supplemented by iodine coated urea fertilizer or foliar application to test agronomic approaches at a large scale to enhance the iodine content of staple crops that are major source of food in Pakistan.

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CONFLICT OF INTEREST

All the authors declare that there is no conflict of interest for this research work.

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Table 1: Summary physico-chemical properties of soils used in the study

Sample no.	pH (1:2.5)	Electrical conductivity (1:2.5)	Organic matter	Available P	Extractable K	Extractable Zn	Extractable B	Extractable Fe
		dS m^{-1}	%	µg g^{-1}				
Geometric Mean	8.1	0.29	0.81	5.2	139	0.51	0.57	6.55
Median	8.1	0.27	0.80	5.5	140	0.50	0.60	6.50
Mode	8.1	0.25	0.80	8.0	110	0.50	0.60	5.30
SD	0.3	0.21	0.47	4.1	59	0.43	0.59	1.51
Min	7.0	0.10	0.19	1.0	52	0.20	0.14	4.30
Max	8.7	1.50	2.79	30.0	315	3.00	3.40	9.80

Table 2: Reference material data for iodine measurements

Reference material	TMAH-extracted I (µg g^{-1})	Water-soluble I (µg g^{-1})	Standard deviation	*n*	Certified data for TMAH-extracted I (µg g^{-1})
GSS-2 (chestnut soil)	1.72	0.043		1	1.8 ± 0.2
GSS-3 (yellow-brown soil)	1.33	NA	0.05	6	1.3 ± 0.4
GSS-5 (yellow-red soil)	4.17	0.19		2	3.8 ± 0.5
GSS-7 (laterite)	21.66	1.35	0.13	2	19.3 ± 1.1
GSS-8 (loess)	1.05	0.064		1	1.6 ± 0.5
NIST1573a (tomato leaves)	0.64	NA	0.02	3	0.66 target value for I at BGS, UK lab
GBW08503 (wheat flour)	0.06	NA	0.06	4	NA

N/A: not available

Table 3: Geographic distribution of iodine in soils, irrigation waters, wheat crop and grains samples across Pakistan

	TMAH Soil Iodine	Water-Soluble Soil Iodine	Irrigation samples Iodine	Wheat Leaves Iodine	Wheat Grains Iodine
	(µg g^{-1})	(µg g^{-1})	(µg L^{-1})	(µg g^{-1})	(µg g^{-1})
	Punjab Province				
Geo. Mean	0.54	0.016	9.64	0.23	0.01
Median	0.52	0.016	8.35	0.21	0.01
Min	0.19	0.001	2.00	0.13	0.01
Max	1.56	0.048	49.9	0.47	0.02
St.Dev	0.34	0.012	16.33	0.105	0.004
N	49	49	18	24	43
	Sindh Province				
Geo. Mean	0.57	0.01	16.24	0.19	0.01
Median	0.54	0.013	10.80	0.18	0.01
Min	0.31	0.008	1.70	0.14	0.01
Max	1.42	0.037	348.8	0.27	0.02
St.Dev	0.29	0.008	131.5	0.039	0.005
N	17	17	7	12	14
	Khyber Pakhtunkhwa Province				
Geo. Mean	2.56	0.04	0.76	0.28	0.01
Median	2.68	0.04	0.80	0.31	0.01
Min	1.66	0.02	0.60	0.18	0.01
Max	4.2	0.08	0.90	0.40	0.01
St.Dev	1.32	0.03	0.15	0.11	0.00
N	4	4	3	4	4
	Baluchistan				
Geo. Mean	1.45	0.06	NA	0.13	0.03
Median	0.81	0.03	NA	0.13	0.03
Min	0.39	0.02	NA	0.12	0.03
Max	9.59	0.27	NA	0.14	0.03
St.Dev	5.19	0.14	NA	0.01	N/A
N	3	3	NA	2	3
	Azad Jammu and Kashmir (AJ&K)				
Geo. Mean	0.99	0.01	12.10	0.27	0.01
Median	1.15	0.01	12.1	0.32	0.02
Min	0.22	0.00	12.1	0.14	0.01
Max	2.87	0.02	12.1	0.46	0.02
St.Dev	0.83	0.007	NA	0.177	0.007
N	13	13	1	4	4
	Pakistan				
Geo. Mean	0.66	0.02	8.47	0.22	0.01
Median	0.6	0.02	7.30	0.19	0.01
Min	0.19	0.001	0.60	0.12	0.006
Max	9.59	0.27	348.8	0.47	0.01
St.Dev	1.17	0.030	68.04	0.10	0.03
N	86	86	29	46	68

NA: not available

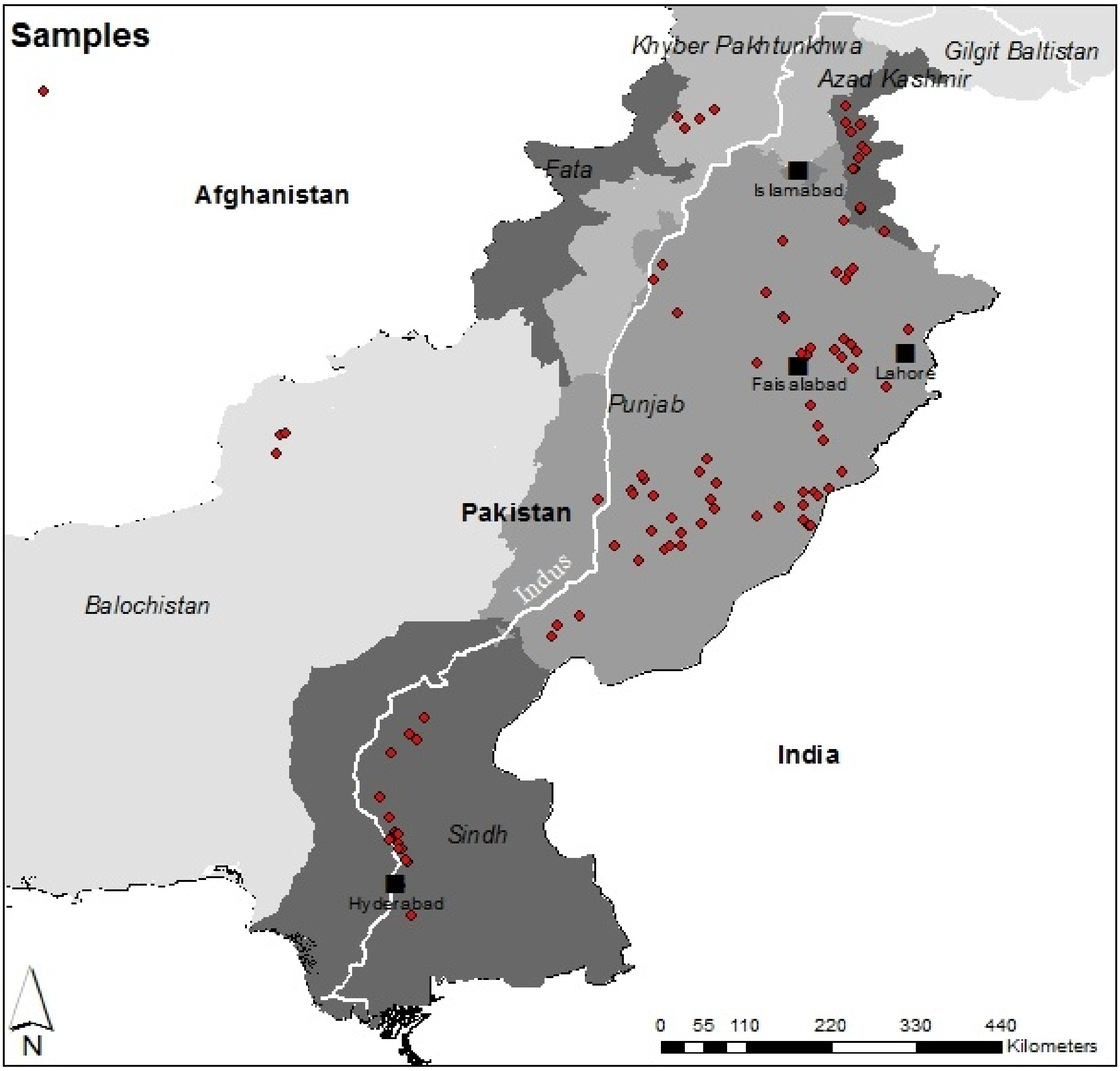

Fig 1. Sampled locations across Pakistan for iodine study

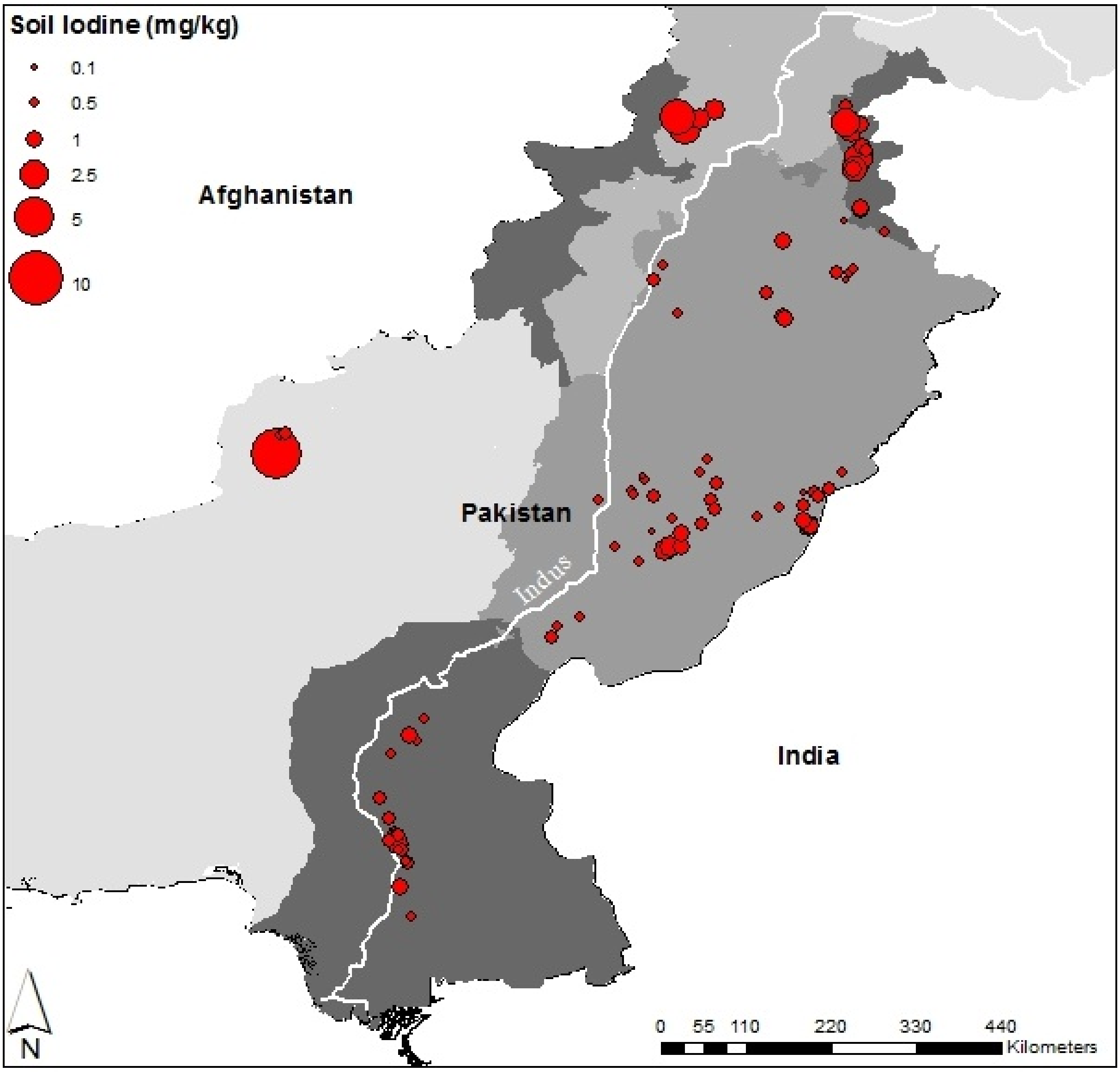

Fig 2. TMAH-extractable soil iodine status of the samples collected across Pakistan

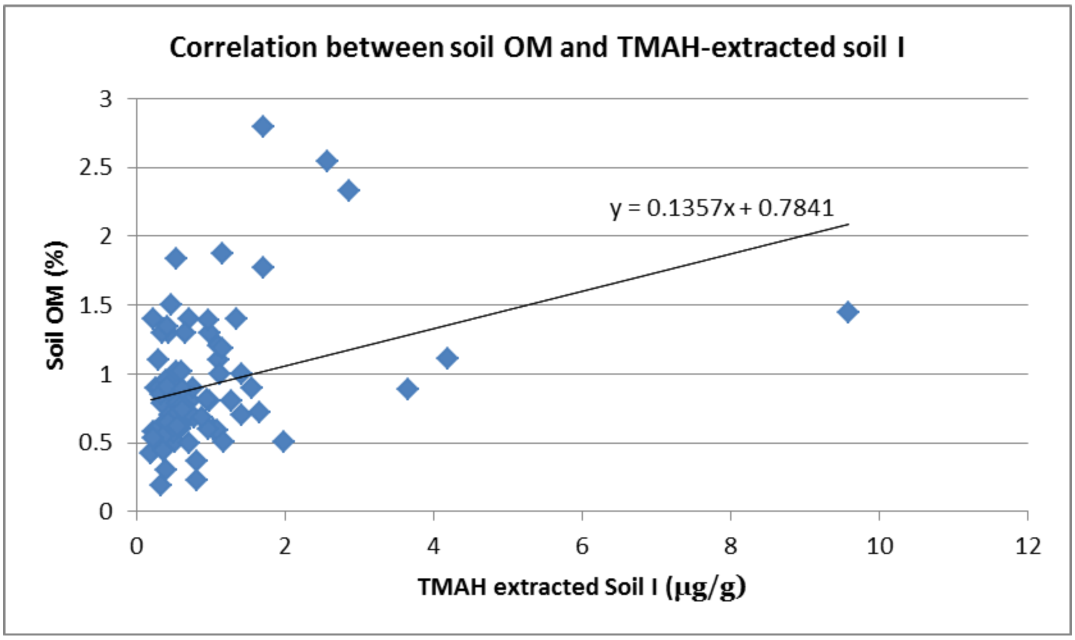

Fig 3. Relationship between TMAH-extractable soil iodine and soil organic matter

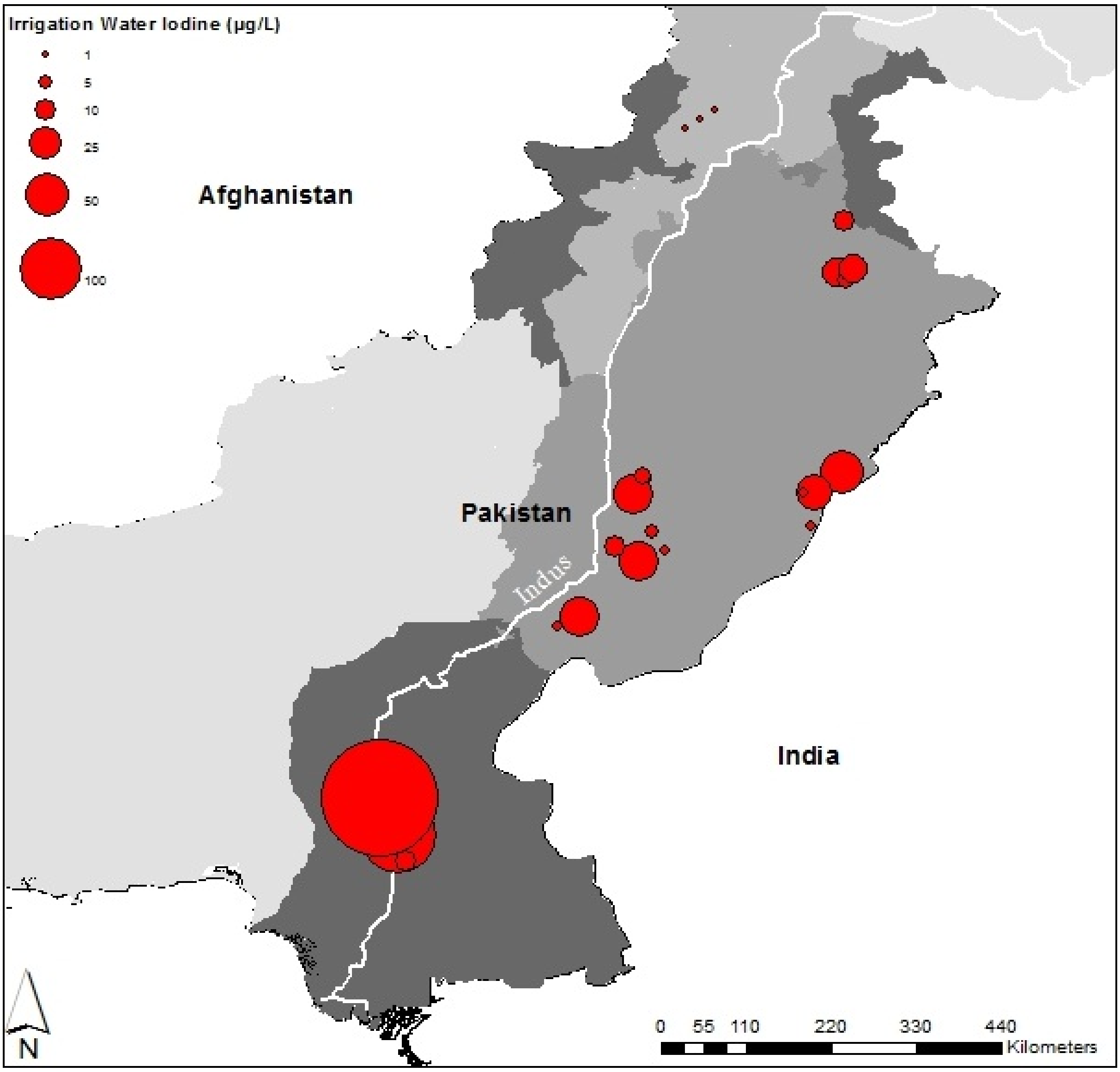

Fig 4. Irrigation water iodine

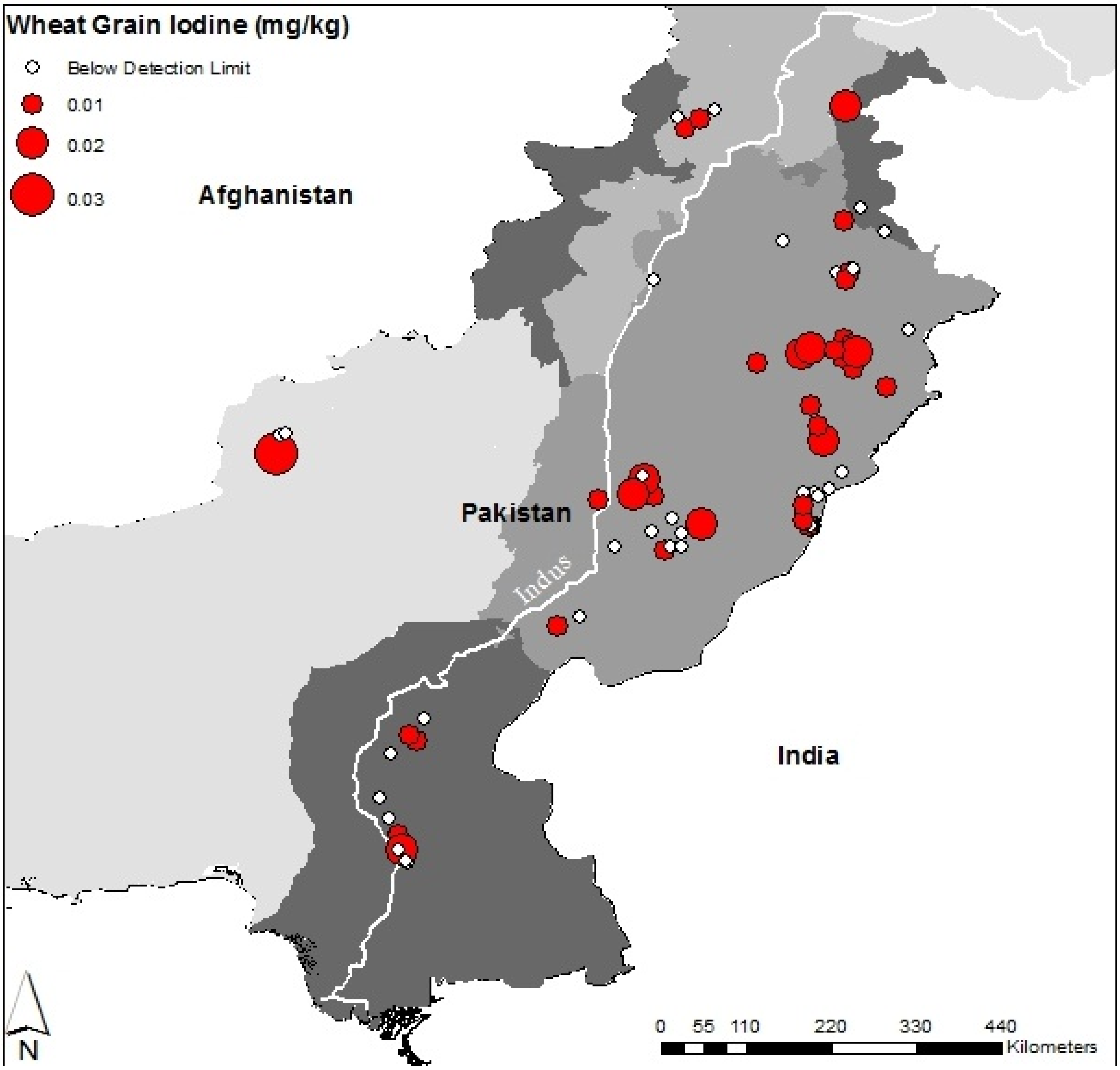

Fig 5. TMAH-extractable iodine in wheat grain samples collected across Pakistan

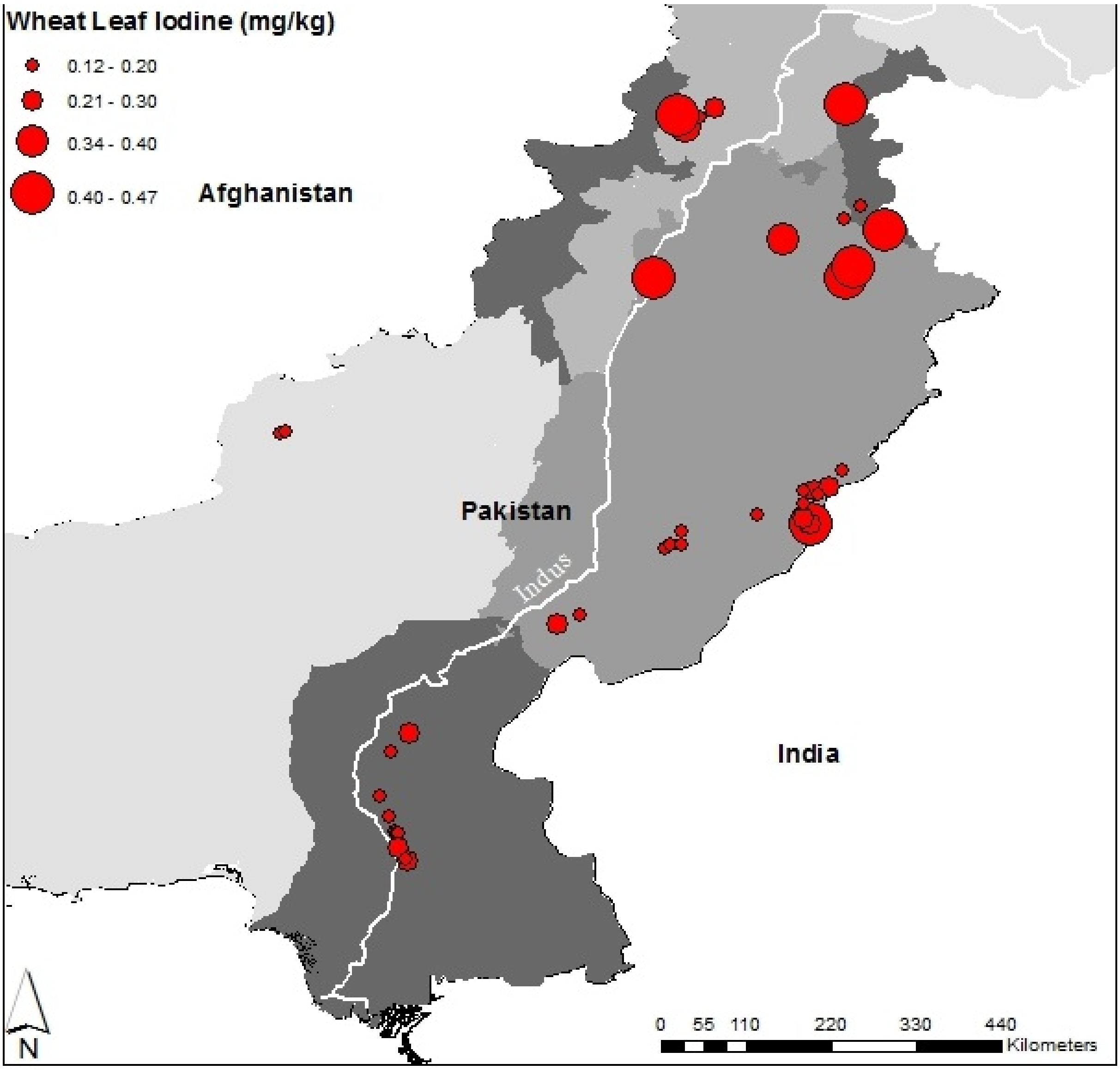

Fig 6. TMAH-extractable iodine in wheat leaves for samples collected across Pakistan

Online Resource 1: Detailed iodine (I) analysis of the soils, grains, leaves and irrigation waters across Pakistan

Lab Serial No.	Location/District	TMAH- I (µg g^{-1})	Water soluble I (% of TMAH-I)	Wheat grain I (µg g^{-1})	Wheat Leaves I (µg g^{-1})	Irrigation water I (µg L^{-1})	Lab Serial No.	Location/District	TMAH Iodine (µg g^{-1})	Water soluble-I (% of TMAH-I)	Wheat grain I (µg g^{-1})	Wheat Leaves I (µg g^{-1})	Irrigation water I (µg L^{-1})
1	Bahawalnagar	0.7	3.5	0.01	0.41	3.0	44	Multan	0.53	2.2	0.01	NA	NA
2	Bahawalnagar	1.34	2.6	0.01	0.24	NA	45	M. Garh	0.39	2.0	0.02	NA	3.6
3	Bahawalnagar	1.1	3.5	BDL	0.23	NA	46	Vehari	0.46	3.4	NA	NA	NA
4	Bahawalnagar	0.43	2.6	BDL	0.21	NA	47	Vehari	0.35	7.0	NA	NA	NA
5	Bahawalnagar	1.1	2.6	BDL	0.26	NA	48	M. Garh	0.33	2.7	BDL	NA	11.5
6	Bahawalnagar	0.29	2.6	BDL	0.15	49.9	49	M. Garh	0.41	3.7	0.02	NA	36.1
7	Bahawalnagar	0.72	2.8	BDL	0.21	34.6	50	Vehari	0.55	2.3	NA	NA	NA
8	Bahawalnagar	0.23	0.6	BDL	0.18	2.0	51	Multan	0.52	3.0	0.02	NA	NA
9	Bahawalpur	1.56	3.1	0.01	0.15	2.0	52	Multan	0.36	2.7	BDL	NA	NA
10	R.Y. Khan	0.36	3.2	0.01	0.28	2.2	53	M. Garh	0.22	4.2	BDL	NA	5.3
11	R.Y. Khan	0.26	6.2	NA	NA	41.8	54	Vehari	0.71	3.2	NA	NA	NA
12	R.Y. Khan	0.47	3.0	BDL	0.16	39.7	55	Vehari	0.62	2.9	NA	NA	NA
13	Bahawalpur	1.41	3.4	BDL	0.19	NA	56	Vehari	0.62	2.7	NA	NA	NA
14	Bahawalpur	1.12	2.9	BDL	0.15	NA	57	M. Garh	0.21	5.7	BDL	NA	6.3
15	Bahawalpur	1.03	2.3	BDL	0.13	NA	58	D.G. Khan	0.34	2.6	0.01	NA	NA
16	Rahimyar Khan	0.76	2.3	NA	NA	NA	59	Mianwali	0.5	3.9	NA	NA	NA
17	Bahawalpur	0.45	4.1	NA	0.17	NA	60	Bhakkar	0.43	3.6	NA	NA	NA
18	Bahawalnagar	0.63	2.0	BDL	0.26	NA	61	Chaar Saddah	3.65	2.1	0.01	0.38	0.9
19	Bahawalnagar	0.51	2.4	BDL	0.13	NA	62	Mirpur, AJ Kashmir	1.09	0.6	NA	NA	NA
20	Bahawalnagar	0.37	9.0	NA	NA	NA	63	Bhimbher	0.26	2.8	BDL	0.46	NA
21	Bahawalnagar	0.98	3.5	0.01	0.21	NA	64	Mirpur, AJ Kashmir	1.28	1.7	BDL	0.14	NA
22	Bahawalnagar	0.65	2.7	0.01	0.16	NA	65	Poonch	2.57	0.9	NA	NA	NA

23	Matiari	0.59	1.7	BDL	0.27	2.9	66	Sandhoti	1.99	0.8	NA	NA	NA
24	Nawabshah	0.61	2.2	BDL	0.18	NA	67	Bagh	1.15	1.4	NA	NA	NA
25	Matiari	1.42	2.6	BDL	0.19	7.3	68	Mardan	1.7	1.7	BDL	0.23	0.8
26	Matiari	0.77	2.9	BDL	0.17	14.5	69	Mardan	1.66	1.2	0.01	0.18	0.6
27	Matiari	0.54	1.4	BDL	0.16	NA	70	M.B. Din	0.63	1.4	BDL	NA	21.7
28	Matiari	0.56	1.6	0.01	0.16	151.4	71	M.B. Din	0.36	2.1	0.01	0.34	5.1
29	Matiari	0.53	1.8	0.02	0.14	1.7	72	M.B. Din	0.19	2.0	0.01	0.41	6.8
30	Matiari	0.5	3.6	BDL	0.25	NA	73	Mianwali	0.71	3.7	BDL	0.42	NA
31	Matiari	0.44	3.0	BDL	0.19	10.8	74	Sargodha	0.96	4.9	NA	NA	NA
32	Kochlaak	9.59	2.8	0.03	NA	NA	75	Sargodha	1.18	2.4	NA	NA	NA
33	Pishin	0.39	5.9	BDL	0.14	NA	76	Chakwal	0.88	3.9	BDL	0.39	NA
34	Pishin	0.81	4.0	BDL	0.12	NA	77	Sargodha	0.55	2.2	NA	NA	NA
35	Khairpur	0.35	3.2	0.01	NA	NA	78	M.B. Din	0.36	2.8	BDL	0.47	18.3
36	Khairpur	0.42	2.3	BDL	NA	NA	79	Mirpur, A J Kashmir	0.22	2.6	0.01	0.17	12.1
37	Nawabshah	0.95	2.1	0.01	0.23	NA	80	Sandhoti	0.97	1.2	NA	NA	NA
38	N. Feroz	0.33	3.0	BDL	0.17	NA	81	Hatian bala	1.71	0.4	NA	NA	NA
39	Nawabshah	0.52	1.9	BDL	0.18	348.8	82	Mirpur, A.J. Kashmir	0.61	1.5	0.02	0.46	NA
40	Hyderabad	0.98	2.9	NA	NA	NA	83	Hatian Bala	1.15	0.4	NA	NA	NA
41	Tando Muhammad Khan	0.31	5.3	NA	NA	NA	84	Bagh	0.53	0.9	NA	NA	NA
42	Jamshoro	0.81	2.1	NA	NA	NA	85	Muzaffarabad	2.87	0.7	NA	NA	NA
43	M. Garh	0.37	2.2	BDL	NA	9.9	86	Chaar Saddah	4.2	1.1	BDL	0.40	NA

BDL: Below detection limit; NA: data not available