Translocation of Aluminum to Grain Crops Grown in Different Agricultural Systems

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Abstract

The aim of this study was to evaluate the mobility and transport of Aluminum (Al) by shoot and grain crops (wheat and maize) grown on two different agricultural soil irrigated with water have high (lake water) and low levels (canal water) of Al. The total and bioavailable fractions (deionized water, 0.11M CH_3COOH, 0.05M ethylenediaminetetraaceticacid (EDTA) and 0.1 M HCl extractable) of Al in both understudied agricultural soils and correlate with respective total Al in the edible parts (grains) and non edible parts (Shoots) of wheat and maize. The Al content in lake and canal water samples was found in the range of 750 – 1340 and 90 – 150 µg/L respectively. The total and extractable Al in both agricultural soil samples, edible and non edible parts of wheat and maize were analysed by atomic absorption spectrometry after acid digestion in microwave oven. The edible and non edible part of both crops absorbed significantly high levels of Al grown on agricultural soil irrigated with lake water (SILW) as compared to those grown on soil irrigated with canal water (SICW) had low level of Al (p<0.01). The transfer factor of Al from soils to edible and non edible parts of wheat and maize were also evaluated. It was observed that the bioaccumulation of Al was found to be high in non edible parts of both crops grown in SILW. This study highlights the increased danger of growing food crops in the agricultural land continuously irrigated by Al contaminated lake water.

Keywords: Aluminum; Agriculture soils; Grain crops; Translocation; Water.

Introduction

Aluminum (Al) is the third most abundant element in the earth's crust [1]. Owing to this fact, Al toxicity is one of the most important negative factors constraining crop performance [2, 3]. This problem is enhanced if the soil pH becomes acid, as is now the case on 40% of the arable lands in the world [4]. One of the general aspects of Al toxicity is the inhibition of root growth owing to peroxidase-mediated hydrogen peroxide production [5] and/or loss of plasma membrane integrity and cell division [6, 7]. In terms of resistance at the biochemical level, different metabolites such as organic acids [2, 8] and some phenols [9, 10] have been suggested as potential ligands for Al ions. Recently, considerable attention has been focused on the determination of trace aluminum in biological and environmental samples because aluminum is toxic to plant, aquatic organism and human if absorbed [11].

The impacts and diseases related to aluminum were given in detail in the literature [12]. Interest in the potential link between high aluminum contents in tissues and various neurodegenerative disorders such as Alzheimer's disease has drawn the attentions to the intake of aluminum from food, drinking water, parenteral nutrition or dialysis fluids in individuals with chronic renal disease. Hence, the Joint Food and Agricultural Organization (FAO)-World Health

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Organization (WHO) Expert Committee on Food Additives established the provisional tolerable weekly intakes (PTWI) for adults of 7.0 mg Al kg^{-1} of body weight [15].

However, the toxicity of Al depends not only on total concentration but also on its chemical forms. On the other hand, identification of chemical forms or phases of Al in soil is necessary for estimating its biological availability, physicochemical reactivity, and transport in the environment and into the food chain. In unpolluted soils, trace metals are mainly bound to silicates and primary minerals forming relatively immobile species, whereas trace metals in polluted soils are generally bound to other soil phases being more mobile. The partitioning of a metal among various soil phases gives more complete information on its mobility as well as on bioavailability or toxicity. Therefore, the fractionation is performed by extraction of soil with chemical reagents or solvents that extract the elements bound in, or associated with, a particular soil phase or component [16]. The sequential extraction was also used for the speciation of metals in soil [17-19]. Analytical methodologies for aluminum speciation in environmental and biological samples were reviewed in recent times [20, 21].

To date, no methods have been considered as universally applicable for the assessment of metal fractions in soils and plant bioavailability. Most of data derived from bioavailability studies tend to be inconsistent, making interpretations difficult [22-24]. The reasons for this fact are that metal bioavailability depends on many factors such as plant species and soil properties. Different fractions of soil metals vary considerably in their chemical reactivity and bioavailability [23, 25-27]. In addition, the present approach for bioavailability prediction does not take into account the real chemical speciation of metals in soils and uptake mechanisms of metals by plants either. The main reasons for this are the inherent difficulties in obtaining the detailed information on the biological active speciation of metals in soils and uptake through biological membrane of plants. Considering this situation one can understand why there are no universally experimental methods available for prediction of bioavailability of metals in soils in the literature [28] (Sauve et al., 2000).

The used methods for the determination of Al in environmental and food samples generally include the results obtained by flame and flameless atomic absorption spectrometry [29-33]. It is recognized that because flame atomic absorption spectrometry (FAAS) is faster, cheaper, and does not require expert operators.

The aim of present work was to determine the total Al in lake and canal water. The transport of Al to shoots and grains of wheat, and maize through soils irrigated with lake and canal water were also evaluated. The extractable Al in water, acetic acid, HCl, EDTA and total Al in soil samples, both parts of wheat and maize, were determined by FAAS using nitrous oxide – acetylene flame. The correlation coefficients between total concentrations of aluminum in grain samples and soil-extracts were found in order to find the extraction agent that represents the extractable proportion of aluminum from soil to plant.

Experimental
Samples collection and treatment

During 2008 to 2009, the lake water (n = 24), canal water samples (n = 24), and two batches of soils, maize and wheat plants (grains and shoot) samples were collected from two different agricultural fields randomly from Bobak and Jamshoro, Sindh Pakistan. One agricultural field was known to be control samples, i.e. soil irrigated with canal water contains low level of Al contents. The other as test samples, agricultural soil irrigated with Al contaminated lake water. The surface water samples of different origin (lake and canal) were collected by using Van Dorn plastic bottles (1.5 L capacity) and was kept in well stoppered polyethylene plastic bottles previously soaked in 10% nitric acid for 24 h and rinsed with ultra pure water. All water samples were stored in insulated cooler containing ice and delivered on the same day to laboratory and all samples were kept at 4 °C until processing and analysis.

The edible (grains) and nonedible (shoots) parts of wheat (*Triticum aestivum*, L.) and maize (*Zea mays* L.) were collected from two different agricultural fields randomly from Bobak and Jamshoro, where agricultural soil irrigated with

lake water (SILW), as test samples (TS). The same grain samples were collected from agricultural soil, irrigated with canal water (SICW) as control samples (CS).

The surface layer of soil samples (0-25 cm) with a stainless steel auger were collected from the same locations simultaneously with the grains. On returning to laboratory, soil samples were spread on plastic trays in fume cupboard, air dried for eight days at room temperature. The all edible and non edible parts of both crops were put through a three steps of washing sequence, which involved agitating and rinsing first with distilled water followed by three separate washes in ultra pure water. The washed grains and shoots of both crops samples were air dried, weighed and placed in an electric oven at 85 ºC for 48 hours. The dried wheat and maize samples were homogenized by grinding in an agate mortar and sieved through a nylon sieve (<125 µm) mesh size. The final samples were kept in labeled polypropylene containers at ambient temperature before analysis.

Reagents and glassware

Ultrapure water obtained form ELGA lab water system (Bucks, UK), was used throughout the work. Ammonium acetate was purchased from Sigma (Aldrich, Milwaukee, USA). Acetic acid (glacial 100%), Hydrochloric acid (65%, sp. gr. 1.4), nitric acid (37% sp. gr.1.19), and hydrogen peroxide (30%) were of analytical reagent grade from Merck (Darmstadt, Germany). Standard solution of Al was prepared by dilution of 1000 mg/L certified standard solution, Fluka Kamica (Buchs, Switzerland). Extractant solution 0.05 mol/L, EDTA at pH 7 was prepared by dissolving disodium dihydrogenethylenediaminetetraacetate salt dihydrate (Na_2 EDTA$\times$2$H_2$0 Merck). The pH solution was adjusted to 7.0 by adding NH_4OH solution (trace element quality, Fisher). The certified reference material BCR 483 (soil), BCR 100 (beech leaves) and BCR 101 (spruce needles) were purchased from the Community Bureau of Standards (BCR, Brussels, Belgium). All glassware and plastic material used was previously treated for 24 h in 2 N nitric acid and rinsed with double distilled water and then with ultrapure water. We used 50 mL of acid washed polyethylene centrifuge tubes for extraction, while 50 mL polyethylene vessels Bibby (Sterilin Ltd., UK) were used for storage of extractants.

Apparatus

A centrifuge ROWKA Laboratoryjna type WE-1, nr-6933 (Mechanika Phecyzyjna, Poland) was used for separating solid phase. A domestic microwave oven (PEL PM023 Osaka, Japan), programmable for time and microwave power from 100 to 900 W, was used for acid digestion of samples. A pH meter (Ecoscan Ion 6, Kuala Lumpur, Malaysia) was employed for pH adjustments and pH determination. The determination of Al in all extracts and digests of understudy samples were carried out by means of a double beam Perkin Elmer atomic absorption spectrometer model AA700 (Norwalk, CT, USA) equipped with flame burner at 12.5 mm height using nitrous oxide – acetylene flame. Single element hollow cathode lamp for Al was operated At 12 mA with a spectral bandwidth of 1.3 nm. The analytical wavelength was set at 309.2 nm. The composition of flame, Fuel (acetylene 0.45kg/cm), oxidant (Nitrous oxide 1.6 kg).

Analytical procedures
Extraction of Al by 0.1M HCl

Weighed duplicate samples of soil and replicate six samples of certified soil sample (BCR483) in extraction bottles and added 25 mL of HCl 0.1 mol/L. The mixture was shaken in a mechanical end-over-end shaker at a speed of 30 rpm for 1 h at room temperature. The extract was separated by centrifuging at 3000 rpm, and the supernatant liquid was filtered through Whatmann 42 filter paper, and stored in polyethylene bottles at 4 °C until analysis [34].

Extraction of Al by 0.05 M EDTA

Weighed duplicate 0.5 g of soil samples of each batch directly in the extraction bottles (250 mL polypropylene bottles) and added 50 mL of 0.05 M EDTA. The mixture was shaken in a mechanical end-over-end shaker at a speed of 30 rpm for 1 h at room temperature. The extract was separated by centrifuging at 3000 rpm, and the supernatant liquid was filtered and stored in polyethylene bottles at 4 °C until analysis [35, 36].

Leaching test (DIN 38414-S4)

Duplicate samples of each batch of air-dried soil samples (1g) were weighed into extraction bottles. Added 20 mL of ultrapure water and shaken on a mechanical end-over-end shaker at a speed of 30 rpm for 1 h at room temperature. The extract was separated by centrifuging at 3000 rpm, and the supernatant liquid was filtered through Whatman 42 filter paper, and stored in polyethylene bottles at 4 °C until analysis.

Extraction of Al by 0.11M CH₃COOH

Weighed duplicate 0.5 g of each batch of soil samples directly in extraction bottles (250 mL polypropylene bottles) and 20 mL of 0.11 M acetic acid were added. The content of the extraction bottles were shaken in a mechanical end-over-end shaker at a speed of 30 rpm for 1 h at room temperature. Then each extract was separated by centrifuging at 3000 rpm, and the supernatant liquid was filtered and stored in polyethylene bottles at 4 °C until analysis [37].

Physicochemical studies

The physicochemical parameters for soil samples of both agricultural fields pH, organic matter (OM), and cation exchange capacity (CEC) were determined by using standard methods [38]. The pH was determined for each batch, using a ratio of soil to ultra-pure water of 1:2.5 (w/v) [39]. The OM content was obtained by ashing triplicate samples of each batch in muffle furnace at 540 °C for 6 h. The change in the dry weight of soils before and after ashing was used to calculate the OM content [40]. The CEC were determined by ammonium acetate at pH 7 using standard methods [41, 42]. All analysis was performed in triplicate. Blanks were run simultaneously.

Results and Discussion
Quality control

The linear range of the calibration curve reached from the detection limit up to 500 µg/L for Al. The data revealed excellent coefficient of correlation coefficient, 0.998. The detection limit (LOD) was defined as 3 s/m, where s is the standard deviation corresponding to 10 blank

injections and 'm' is the slope of the calibration graph, obtained for each element. The LOD of 2.4, µg/L was calculated for Al. The reliability of microwave assisted acid digestion for estimation of Al, was checked by certified reference materials have different matrixes, SRM 1643e (water), BCR 483 (soil), BCR 100 (Beech leaves) and BCR 101 (Spruce needles). The obtained results were summarized in (Table I). It can be seen that the recovery value was >97%.

Table 1. **Determination of Al in certified reference materials (mg/kg / µg/L).**

Certified	Obtained value	Certified values /Literature values
SRM 1643e (Water) µg/L	138 ± 4.2	141.8 ± 8.6
BCR 483		
Acetic acid	52.5 ± 3.1	50.1 ± 2.6 [a]
	----------	50 ± 2 [b]
HCl	2562 ± 44	2530 ± 42 [c]
Total	15569 ± 123	15422 ±148
BCR 100 (Beech leaves)	432 ± 5.68	435 ± 4.00
BCR101 (Spruce needles)	173 ± 4.26	172.97 ± 4.19

[a] Matus et al., 2004, [b] Sutherland and Tack, 2002, [c] Sutherland and Tack, 2008, Kubova et al., 2008.

Aluminum in water samples

The concentration of Al in canal water samples was observed in the range of (90 - 150 µg/L) which are within WHO permissible level [43] (WHO, 1997) (Table 2), while in lake water samples high content of Al was found in the range of (750-1340 µg/L). This can be attributed to reduction in precipitation, surface wastage runoff with rainwater into understudy Lake and increasing rate of evaporation during summer [44]. All this provides evidence that anthropogenic and geological environment play a key role in the distribution of Al in lake water samples. Number of epidemiological studies showed an association between Al in drinking water and Alzheimer's disease, an important form of senile dementia in man [45, 46]. Aluminum toxicity is one of the most deleterious factors for plant growth in acidic soils because over 50% of the world's potentially arable lands are acidic [47]. In recent years, there has been significant progress in our understanding of the physiological responses and tolerance of plants to Al toxicity.

Table 2. **Physico-chemical characteristics of the soil samples irrigated with Lake water (SILW) and soil irrigated with canal water (SICW).**

Parameters	SILW	SICW
pH of water samples	7.10 - 8.20[a]	7.1 - 7.6
pH of soils	7.4 - 8.6	7.2 – 8.3
Organic matter (%)	24.7 ± 2.23[b]	23.8 ± 2.13
Sand (%)	42.5 ± 3.12	41.0 ± 2.5
Silt (%)	32.1 ±1.84	21.2 ± 1.38
Clay (%)	3.4 ± 0.67	2.9 ± 0.58
CEC(mequiv./100 g)	14.3 ± 1.2	14.0 ± 2.40
Al in water samples (µg/L)	750 - 1340	90 - 150
Water soluble (mg/Kg)	45.25 ± 3.89	36.0 ± 1.41
0.11M acetic acid (mg/Kg)	357.50 ± 17.68	241.5 ± 36.06
EDTA (mg/Kg)	138.5 ± 9.19	108 ± 8.49
HCl (mg/Kg)	704.5 ± 21.92	653 ± 50.91
Total Al (mg/Kg)	19900 ± 77.8	16000± 117.4

Key: [a] (range)
[b](mean± standard deviation)

Bioavailable fractions of Al in soil

To know the potential risk of Al to plants, animals and human beings, it is necessary to evaluate its mobile and/or available fractions in control and contaminated soils. Researchers have been tried to measure the plant-available fraction of metals in soils using different extraction procedures. The mobility of trace metals, their bioavailability and related eco-toxicity to plants, depend strongly on their specific chemical forms or ways of binding [48, 49]. The lixiviation of metals from soils using selective extractants gives valuable information, especially for agricultural purposes.

The two reagents validated by a group of European researchers coordinated by the Measurements and Testing Program of the Commission of the European Community, in single extraction procedures [50], are EDTA 0.05 M, in either the di-sodium or di-ammonium salt form, has been used extensively as an extractant of potentially plant available metals. In some trials, EDTA was found to give a very good indication of the toxic metals pollution hazard in soils as well as being a reliable test for predicting plant-available metals [51]. Neutral salt extractants are generally weaker extractants than EDTA and

give an indication of the immediately exchangeable (therefore immediately plant-available) metals.

In this work, deionized water, 0.05M EDTA (pH 7), 0.11M CH₃COOH and 0.1M HCl were chosen as the extracting solutions. The extractable Al concentrations in CS and TS samples are listed in (Table 2). The percentages of water extractable Al relative to the total content was found in the range of 0.2 -0.25%) in both soil samples. Results shows that the available fractions of Al were high in TS samples as compared with those obtained from CS samples. In fact, there were statistically significant correlations of total concentrations of Al in both soil samples with those obtained in all extractants was found in the range of   (R^2 = 0.56 - 0.81).

Predicting the uptake of Al by wheat and maize crops

Our finding reports that, the levels of Al in lake water samples was exceeded the maximum permissible level of Al (200 µg/L) recommended by WHO for drinking purpose while canal water samples was within permissible level (Table 2). Total Al concentrations in edible and non edible parts of wheat and maize grown on TS were found in the range of (32 - 46 and 323 - 398 mg/kg) and (31 - 40 and 308 - 351 mg/kg) respectively. Whereas, the total concentrations of Al in edible and non edible parts of wheat and maize grown on CS were found in the range of (19 - 30 and 206 – 225 mg/kg) and (17 - 26 and 223 - 256 mg/kg) respectively, on dry weight basis. The results shown in (Tables 3) indicate that a high level of Al was observed in non edible part of wheat and maize than edible part grown in both agricultural fields. The levels of Al in shoots and grains of wheat and maize grown in TS were significantly higher than those values observed in edible and non edible parts of both crops grown in CS (P < 0.01). It is described that metal concentrations of the EDTA-extracts of soil are commonly used to indicate the availability of metals for plant uptake [16]. It can be seen from (Table 4), that the correlation coefficient (r=0.89) between Al in both crops and in the EDTA extract is higher than other extractants and total Al contents (p<0.05). Therefore, both studied crops grown on TS showed

the high accumulation of Al, might be due to its elevated concentration in irrigated water and soil. It is predicted that Al contaminated crops affect food quality and subsequently human health through contamination of the food chain. The non edible parts of wheat and maize are commonly used as animal fodder [52], which may effect the animal health. The wheat is main cereal cultivated in Pakistan and covers about 80% of total cereal cropped area and largely used as human diet, whereas, grains of maize were used as major contributor in dairy and poultry.

Table 3. **Translocation of Al to wheat and maize plants grown in soil irrigated with lake (SILW) and canal water (SICW) (mg/Kg on dried basis).**

Samples	SILW	SICW	Transfer factor (T_f) [a]	
			SILW	SICW
Wheat(grains)	38.6 ± 4.82	23.9 ± 3.5	0.28	0.22
Wheat Shoot	373 ± 20.8	216 ± 6.61	2.69	2.00
Maize (grains)	35.3 ± 2.64	20.6 ± 2.67	0.26	0.19
Maize Shoot	328 ± 12.21	235 ± 10.05	2.37	2.18

[a]Transfer factor (T_f) = Total Al in crops/EDTA extractable Al in soil

Table 4. **Correlation coefficients between the aluminum concentrations in crops and in soil extracts.**

Sample	Total	0.5M HCl	0.05M EDTA	0.11M CH$_3$COOH	Water slouble
Crops grown by Lake water					
Wheat grains	0.1095	0.5086	0.7198	0.0096	0.0408
Wheat Shoot	0.1832	0.7003	0.8956	0.1268	0.174
Maize grains	0.1726	0.7564	0.8959	0.0953	0.163
Maize Shoot	0.2132	0.7118	0.8761	0.1229	0.2028
Crops grown by Canal water					
Wheat grains	0.7505	0.6307	0.8809	0.9561	0.8802
Wheat Shoot	0.7362	0.6808	0.7905	0.9353	0.9091
Maize grains	0.7479	0.5017	0.7145	0.9135	0.8139
Maize Shoot	0.6966	0.6027	0.8898	0.9765	0.8207

Conclusion

This study highlights the potential accumulation of high levels of Al in edible and non edible parts of wheat and maize grown in agricultural soil irrigated with Al contaminated lake water. The contamination of shoots and grains of both crops was apparent in samples obtained from the contaminated soil than those collected from CS. The bioavailable fraction of Al in soils using different extractants including EDTA would help in understanding of soil plant relationships regarding metal uptake. Such a relationship is rarely observed between the total soil content of a given metal and that of the plant. The water samples of lake contain 5 to 6 time higher Al content than permissible limit. From our study it is evident that the water samples of polluted ecosystem may due to anthropogenic and domestic waste. The high availability of Al contents shows that the contaminated soil needs remediation. It can be presumed that the available portions of Al can be reduced by raising pH using lime.

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