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Cobalt Concentration in Rice Cultivars and Soil Irrigated with Untreated Wastewater

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Abstract: The assessment of quality of untreated wastewater is prerequisite before its application for crop husbandry. A field survey was organized to assess cobalt (Co) concentration in wastewater, irrigated rice cultivars and soil. Plant tissue and soil samples were collected from experimental field of Nuclear Institute of Agriculture (NIA), Tando Jam, where the untreated wastewater is used for irrigation. For comparison, water, rice crop and soil samples were also collected from canal water irrigated field. In wastewater irrigated field, three rice cultivars (Shandar, Shua-92 and Sarshar) and in canal water irrigated field, one rice cultivar (Shandar) was grown. The irrigation water analysis results indicate that the EC was above permissible limit for irrigation and pH was strongly alkaline in reaction. The Co concentration in wastewater (0.11±0.02 mg L^{-1}) and canal water (0.15±0.01 mg L^{-1}) was high and unfit for irrigation. The highest Co concentration in paddy (12.32±0.77 mg kg^{-1}) and straw (9.43±1.97 mg kg^{-1}) was found in cultivar Shandar irrigated with canal water and Shua-92 (12.13±0.76 mg kg^{-1}) irrigated with wastewater. The cultivar, Sarshar accumulated the least Co in its straw and paddy (5.93±1.29 mg kg^{-1} and 5.40±0.26 mg kg^{-1} respectively). Cobalt concentration in soils irrigated with canal water and wastewater was more or less equal at both soil depths (0-15 cm and 15-30 cm) and ranged from 23.53±2.30 to 30.24±1.82 mg kg^{-1}. These values are above the Target value for cobalt in soil (9.0 mg kg^{-1}). It is suggested that the wastewater should not be used for irrigation in the selected area because of its high levels of Co, pH and EC, and subsequently build-up of Co in rice tissues and soil. Canal water should be monitored to find out the source of Co input, and its discharge into canal water should be minimized.

Keywords: Cobalt, wastewater, rice tissues, soil

1. INTRODUCTION

Pakistan has become a water-stressed country due to depleting surface and ground water resources (Qadir *et al.,* 2007; Khan *et al*., 2013). Therefore, search for non-conventional water resources for irrigation is an immediate requirement. Non-conventional water resources include brackish groundwater, desalinized seawater, harvested rainwater, wastewater and agricultural drainage water and saline groundwater for irrigation (Qadir *et al.,* 2007).

Farmers use wastewater for irrigation because of many reasons including lack of irrigation water availability, a rich source of plant nutrients, savings of fertilizers and organic matter expenditures, increase in crop yield, improvement in soil properties, conservation of available water, and lack of infrastructure and facilities for safe disposal of wastewater (Jiménez, 2006; Nawaz *et al.,* 2006; Qadir *et al.,* 2007). However, this wastewater, if used untreated, becomes a source of many organic and inorganic pollutants.

In Pakistan, the urban agricultural soils are often irrigated with city effluents for growing crops (Khan *et al*., 2013; Randhawa *et al*., 2014). The continuous use of wastewater in irrigation without a proper management may convert the fertile soil to a contaminated soil. This practice of wastewater irrigation builds up pollutants in soils and plants which would ultimately affect animal and human health. Among various inorganic pollutants, cobalt (Co) has been reported to exist in wastewater, soil and plant samples that have been collected across various regions of Pakistan (Fardous *et al.,* 2011; Shar *et al.,* 2013; Randhawa *et al*., 2014; Amin *et al.* 2014).

Research indicates that the cobalt is mainly required for the growth of legumes and pastures, ruminants' health and microorganisms (Palit and Sharma, 1994; Collins and Kinsela, 2011). In legume crops, Co fixes the molecular nitrogen in root nodules (Palit and Sharma, 1994). Cobalt is not essentially required by higher plants; however, positive effects of this element in higher plants have also been reported. The positive effects include retardation of leaf senescence, drought resistance in seeds, regulation of alkaloid accumulation, and inhibition of ethylene biosynthesis (Palit and Sharma, 1994). In contrast, the negative effects of Co have also been described in the literature whereby a decrease in growth and yield, and changes in morphology, physiology and cytology of various plant species was observed (Palit and Sharma,

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1994) In rice, the exogenous addition of 25 to 50mg kg^{-1} of cobalt to the soil was toxic to rice plants (Kitagishi and Yamane, 1981). The concentration of cobalt in plants varies from 0.02 mg kg^{-1} to 10,220 mg kg^{-1}, with a typical concentration of less than 10 mg kg^{-1} (Collins and Kinsela, 2011).

Cobalt concentration in soil is mainly inherited from parent materials, with a worldwide mean value of 10 mg kg^{-1} at surface level (Kabata-Pendias, 2011). Swartjes (1999) proposed a cobalt concentration of 9.0 mg kg^{-1} as Target value for cobalt in soil; value greater than 9.0 mg kg^{-1} pose a potential risk to ecosystems. The essentiality of cobalt for human body was recognized in 1948 (Khurshid and Qureshi, 1984). In humans, the cobalt is an integral component of vitamin B_{12}, helps in prevention and treatment of pernicious anaemia, aids in production of red cells, and supports normal functioning of nervous system (Khurshid and Qureshi, 1984; Sobukola *et al.* 2010).

Considering these facts, a field survey study was conducted to study the buildup of cobalt in soil with respect to depths and to determine the cobalt concentration in rice tissues (paddy and straw) as a function of wastewater irrigation. The findings of present study will provide data to the limited inventory of Co for wastewater, soil and rice plants in Pakistan and will propose the suitability of wastewater and soil for cultivation with respect to cobalt. Cobalt intake by humans was not calculated in this study, because the cultivated rice crop is only used for seeding purpose

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2. MATERIALS AND METHODS

This survey study was conducted at the experimental farm of the Nuclear Institute of Agriculture (NIA, 25º 25′ 16.17″ N, 68º 30′ 51.04″ E), Tando Jam, Pakistan. The main source of irrigation in experimental field of NIA was wastewater (mixture of sewage water and automobiles workshops). The experimental plot size was (75 m x 100 m) where three rice cultivars (Shandar, Shua-92 and Sarshar) were grown with wastewater. Each rice cultivar was grown in four sub-plots (25 m x 20 m). This area has been under wastewater cultivation for many years. For comparison, a canal water irrigated area was selected from Latif farm, Sindh Agriculture University, Tando Jam (25° 26′ 31.48″ N, 68° 33′ 12.29″ E). The size of the subplot from where the canal water irrigated plant and soil samples were taken was 56x 45m. Only Shandar variety was grown there.

Wastewater samples were collected in clean plastic bottles (500 mL) from the channel adjacent to the irrigated field. Two samples of wastewater were collected at a time interval of 10 minutes. Similarly two canal water samples were collected from water channel at Latif farm. These samples were immediately filtered with Whatman filter paper (42) and were determined for EC and pH using the digital EC and pH meters. After that, 1 mL of concentrated nitric acid (HNO_3) was added to the wastewater and canal water samples to control microbial activity (Yadav *et al.,* 2014). These samples were refrigerated before further analysis. Cobalt concentration in irrigation water samples was analyzed using Atomic Absorption Spectrophotometer (AAS, Novaa 400, Analytikjena, Germany) at Soil and Environmental Sciences Division, NIA Tando Jam.

At the time of maturity, rice cultivars were harvested. Five plants of each rice cultivar irrigated with wastewater were collected randomly from each subplot by cutting at the soil surface with sharp sickle. These plant samples were composited to make one sample and were considered one replication of a rice cultivar. Following this sequence, four replications of plant samples were collected for each rice cultivar. In a similar way, rice cultivar Shandar was collected that was irrigated with canal water. The collected rice plant samples were separated into straw and paddy manually and were rinsed with running tap and distilled water to remove soil particles. The samples were dried into hot air oven at 70 °C for 48 hours. Afterwards, straw and paddy samples were ground into powder through grinding mill (Wiley Mill). The powder of these rice tissues was used to determine the concentration of cobalt with wet digestion technique using Atomic Absorption Spectrophotometer. For digestion, the method proposed by Estefan *et al.* (2013) was used. In brief, 1 gram of plant sample was treated with 5 mL of concentrated nitric acid (HNO_3) and was left overnight. On the next day, 10 mL of acid mixture ($HClO_4 : HNO_3$ 1:2) was added and the content was digested on hot plate at 180-200 ^{0}C until the dense white fumes evolved and transparent white contents were left. The blank samples were also prepared (no plant material in the flask). After cooling, mixture was filtered and volume was made up to 50 mL using distilled water.

After rice harvest, soil samples were collected from each subplot at two depths, 0-15 and 15-30 cm by stainless steel auger. Cobalt concentration in soil was determined by wet digestion method as proposed by Estefan *et al.* (2013). Briefly, 1 gram of air dried soil along with 3 mL of concentrated HNO_3 was added. The content was digested on hot plate at 125 ^{0}C for 1 hour. After that conical flask was removed from the hot plate for few minutes and then added 4 mL concentrated perchloric acid ($HClO_4$) and heated further for 1 hour at 240 ^{0}C. Afterwards, the flask was removed from hot plate and cooled at room temperature. The contents were filtered through Whatman filter paper (42) and the volume was made to 50 mL with distilled water. The data was analysed using descriptive statistics (mean and standard error) for comparison comparison using MS Excel.

3. RESULTS

The data reflected that the EC of canal water samples was 0.66±0.07 dS m^{-1} and of wastewater was 3.67±0.03 dS m^{-1} (Table 1). This suggests that the EC value of wastewater was 6 times higher than canal water samples. The pH of canal water and wastewater was 7.5±0.05 and 8.7±0.10 respectively. The pH of canal water was slightly alkaline and the pH of wastewater was strongly alkaline in reaction. The Co concentration in canal water was 0.15±0.01 mg L^{-1}, and in wastewater, its concentration was 0.11±0.02 mg L^{-1} **(Table 1).** The Co in canal water was 3 times higher than the WWF recommended value of Co in irrigation water (0.05 mg L^{-1}, WWF, 2007). In wastewater, the value was 2.2 times higher than the WWF recommended value.

Table 1. EC, pH and cobalt concentration in irrigation waters

Type of irrigation	EC (dS m^{-1})	pH	Co (mg L^{-1})
Canal water	0.66 ± 0.07	7.5 ± 0.05	0.15 ± 0.01
Wastewater	3.67 ± 0.03	8.7 ± 0.10	0.11 ± 0.02

Each value is a mean ± SE (*n* = 2)

The results regarding Co concentration in paddy and straw are shown in Figure 1 and 2 respectively. Cobalt concentration in the paddy of canal water irrigated rice cultivar (Shandar) was 12.32±0.77 mg kg^{-1}. In contrast, the Co concentration in the paddy of wastewater irrigated cultivars, Shua-92, Shandar and Sarshar, was 12.13±0.76, 7.78±0.44 and 5.93±1.29 mg kg^{-1} respectively. This data suggests that the highest Co concentration in the paddy was found in canal water irrigated rice cultivar Shandar and wastewater irrigated rice cultivar Shua-92. The lowest Co concentration was recorded in the paddy of Sarshar cultivar that was irrigated with wastewater. For Co concentration in straw, the highest Co concentration was recorded in canal water irrigated rice cultivar, Shandar (9.43±1.97 mg kg^{-1}). Among wastewater irrigated rice cultivars, the Co concentration in the straw of Shandar was 6.78±2.43 mg kg^{-1}, Shua-92 was 6.43±2.57 mg kg^{-1} and Sarshar was 5.40±0.26 mg kg^{-1}. Overall, the lowest cobalt was recorded in the straw of wastewater irrigated Sarshar cultivar.

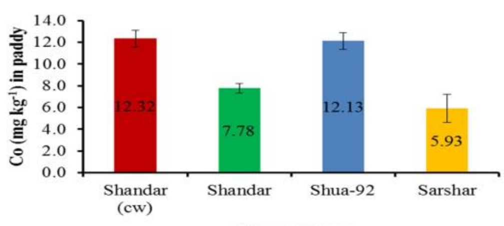

Fig.1. Cobalt concentration (mg kg^{-1}) in paddy of rice cultivars irrigated with canal water and wastewater; each bar is mean ± SE (*n* = 4); cw = canal water irrigated

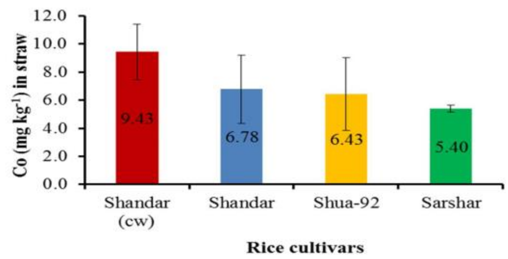

Fig.2. Cobalt concentration (mg kg^{-1}) in straw of rice cultivars irrigated with canal water and wastewater; each value is a mean ± SE (*n* = 4); cw = canal water irrigated

Cobalt concentration in soils that were irrigated with canal water and wastewater are given **in (Fig. 3 and 4).** In the plots of Shandar cultivar, irrigated with canal water, Co concentration of soil was 23.53±2.30 mg kg^{-1} at the depth of 0-15 cm and 26.38±2.41 mg kg^{-1} at the depth 15-30 cm. In wastewater irrigated plots where Shandar, Shua-92 and Sarshar cultivar were grown, cobalt concentration of soil was 24.56±1.78, 30.24±1.82 and 26.65±0.92 mg kg^{-1} at the surface level and at subsurface level the Co concentration was 26.21±2.02, 28.33±2.76 and 26.94±2.76 mg kg^{-1}. At 0-15 cm and 15-30 cm depth, the highest Co was found in plots where the wastewater irrigated rice cultivar Shua-92 was grown.

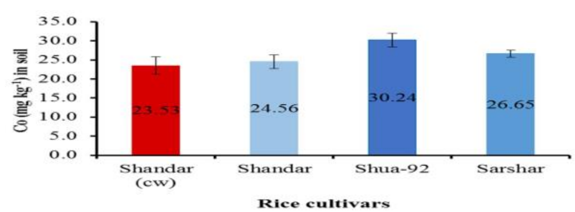

Fig.3. Cobalt concentration (mg kg^{-1}) in soils (0-15 cm depth) irrigated with canal water and wastewater; each bar is a mean ± SE (*n* = 4); cw = canal water irrigated

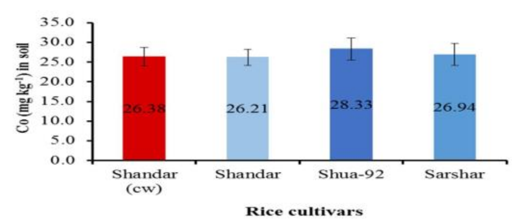

Fig. 4. Cobalt concentration (mg kg^{-1}) in soils (15-30 cm depth) irrigated with canal water and wastewater; each bar is a mean ± SE (*n* = 4); cw = canal water irrigated

4. DISCUSSION

In the present study, the EC of wastewater samples was observed high and the EC of canal water was low. The EC of wastewater sample was above the WWF standards for irrigation (1.5 dS m^{-1}, WWF, 2007). The increased EC in wastewater indicates the high amounts of soluble salts which may be coming from the routine use of various salts in household's chores e.g. NaCl, Na_2CO_3, and $NaHCO_3$. The high EC in wastewater has been previously documented by many researchers in Pakistan. For example, Lone *et al*. (2003) found high level of EC in sewage water (3.2 dS m^{-1}) of Hassanabdal, Attock which was above the acceptable level. Murtaza *et al*. (2003) reported high EC (3.7 to 4.1 dS rn^{-1}) in city effluent samples of Faisalabad.

The pH of wastewater was higher than the canal water. The pH of wastewater sample was strongly alkaline and was above the maximum recommended value for irrigation water (6.5-8.4, WWF, 2007). The possible reason for high pH in wastewater could be high concentration of carbonate (CO_3^{-2}) and bicarbonate (HCO_3^-) ions (Brady and Weil, 2002). Saif *et al*. (2005) found high pH in effluent samples in Korangi area of Karachi. Fazeli *et al.* (1998) observed that the pH in effluent water samples was 8.6 and in unpolluted water samples was 7.4 around the Nanjangud, Mysore District, India.

The Co concentration was slightly higher in canal water as compared to the wastewater samples. However both irrigation waters (canal water and wastewater) samples were higher than WWF recommended value for irrigation (0.05 mg Co L^{-1}, WWF, 2007). A high concentration of Co in wastewater and canal waters of Pakistan has been documented previously. Farid (2003) reported the cobalt concentration in sewage water samples of Attock area above the acceptable limit for irrigation. Wattoo *et al*. (2006) obtained high concentration of cobalt in Phulali canal water of Hyderabad (0.151 mg L^{-1}); they suggested that this water is unsuitable for drinking and irrigation purpose
The highest Co concentration was found in the paddy of Shandar rice cultivar irrigated with canal water and Shua-92 irrigated with wastewater while the lowest Co in the paddy of Sarshar rice cultivar irrigated with wastewater. In straw, the highest Co concentration was also recorded in canal water irrigated rice cultivar (Shandar), while the lowest cobalt was in the straw of wastewater irrigated Sarshar cultivar. Cobalt concentration in paddy and straw reported in present study is within the range described earlier for rice tissues in other countries. Fazeli *et al*. (1998) observed that the Co in rice ranged from 11-21 mg kg^{-1} in straw and from 2-8 mg kg^{-1} in paddy from polluted region of Mysore, India. Cobalt concentration in rice plant shoots has been reported in the range of 1.33 to 7.67 mg kg^{-1} from industrial area in Esfahan city, Iran (Hoodaji *et al.* 2010). However, the Co concentration reported in current study is relatively higher than other studies related to Co in Pakistan that report low levels of Co in vegetables and pulses irrigated with wastewater and canal/tube well water. Ismail *et al.* (2014) conducted study in Multan and reported that the vegetables irrigated with canal water were found to have higher cobalt concentration (1.23 mg kg^{-1}) followed by vegetables that were irrigated with sewerage and tube well water. Hussaini *et al.* (2011) collected vegetables and pulses samples from Gujranwala region and Faisalabad, and they found the highest cobalt (0.79 µg g^{-1}) in cabbage. Samples of vegetables were analyzed by Ahmed *et al.* (2014) grown in peri-urban area of Khushab city, Pakistan; they observed that the Co in vegetable samples ranged from 0.64-1.11 mg kg^{-1} that were irrigated with canal and sewage water. Other researchers Randhawa *et al.* (2014) and Khan *et al.* (2015) also reported low levels of cobalt in vegetables irrigated with wastewater and effluent water than our study.

Soil samples collected after harvest of rice cultivars showed that the Co concentration at both depths and irrigation sources ranged from 23.53±2.30 to 30.24±1.82 mg kg^{-1}. This indicates that the Co concentration in both soils is above the proposed Target value of 9.0 mg kg^{-1} (Swartjes, 1999) and may pose the risk to ecosystem. High levels of Co in soils around Pakistan have also been documented previously. In a study conducted by Ahmed *et al*. (2014), the cobalt in soil irrigated with canal water and wastewater ranged from 14.4-25.2 mg kg^{-1}. Ismail *et al.*, (2014) conducted study in Multan and reported that the soils irrigated with canal water were found to have higher cobalt concentration (18 mg kg^{-1}) followed by tube-well and sewerage water samples. Ahmed *et al*. (2015) reported increased levels of Co in sewage water irrigated soils (12.12-15.75 mg kg^{-1}) of selected area in District Jhang, Pakistan.

5. CONCLUSION

The results of the study indicated that in wastewater and canal water the concentration of cobalt was higher than the acceptable limit. The EC and pH of wastewater samples was also above acceptable limits for irrigation. Rice cultivars varied in accumulation of Co in their tissues (grain and straw). Rice cultivar Shandar, irrigated with canal water, accumulated the highest amount of Co in its tissues. In contrast, cultivar Sarshar, irrigated with wastewater, accumulated the least amount of Co in its tissues. In case of soil, the Co concentration in wastewater irrigated soil was ranged from 23.53±2.30 to 30.24±1.82 mg kg^{-1}; these values are above the Target value of Co in soil. It is suggested that irrigation of rice crop with wastewater should be avoided in the study area. It may lead to Co build up in plants and soil, and may ultimately affect to animals and humans. Since canal water also contain high amount of Co, the sources of Co should be identified and its input in canal water should be avoided.

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