International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:13, No:8, 2019

Evaluation of Heavy Metal Concentrations of Stem and Seed of *Juncus acutus* for Grazing Animals and Birds in Kızılırmak Delta

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Abstract**—***Juncus acutus* (Juncaceae) is a perennial wetland plant and it is commonly known as spiny rush or sharp rush. It is the most abundant plant in Kizilirmak grassland, Samsun, Turkey. Heavy metals are significant environmental contaminants in delta and their toxicity is an increasing problem for animals whose natural habitat is delta. The objective of this study was to evaluate heavy metal concentrations mainly As, Cd, Sb, Ba, Pb and Hg in stem and seed of *Juncus acutus* for grazing animals and birds in delta. The *Juncus acutus* stem and seed samples were collected from Kizilirmak Delta in July, August and September. Heavy metal concentrations of collected samples were analyzed by Inductively Coupled Plasma – Mass Spectrometer (ICP-MS). The obtained mean values of three months for As, Cd, Sb, Ba, Pb and Hg of stem and seed samples of *Juncus acutus* were 0.11 and 0.23 mg/kg; 0.07 and 0.11 mg/kg; 0.02 and 0.02 mg/kg; 5.26 and 1.75 mg/kg; 0.05 and not detectable in July respectively. Hg was not detected in both stem and seed of *Juncus acutus*, Pb concentration was determined only in stem of *Juncus acutus* but not in seed. There were no significant differences between the values of three months for As, Cd, Sb, Ba, Pb and Hg of stem and seed samples of *Juncus acutus.* The obtained As, Cd, Sb, Ba, Pb and Hg results of stem and seed of *Juncus acutus* show that seed and stem of *Juncus acutus* may be safely consumed for grazing animals and birds regarding to heavy metals contamination in Kizilirmak Delta.

Keywords**—**Heavy metals, *Juncus acutus*, Kizilirmak Delta, wetland.

I. Introduction

HEAVY metals substantially contaminate environments, toxicity of heavy metals is an increasing problem for ecological, nutritional and environmental aspects. Heavy metal uptakes are not in line with the increasing concentrations. Many factors affect uptake of metals such as temperature, soil pH, soil competition between the plant species, the type of plant, the root system, the availability of the elements in the soil, soil moisture and plant energy supply to the roots and leaves [1]. Because of the influence of environmental factors, the levels of heavy metals differ in plants [2]. Plants are constant and roots of a plant are the first contact place for heavy metals. Plant stem is exposed to these metals in aquatic systems. Heavy metals are absorbed directly to the leaves since particles remain on leaf surfaces [3]. Wetland plants can accumulate heavy metals in their tissues. For instance, duck weed (*Lemna minor*) and water hyacinth (*Eichhornia crassipes*) are excellent accumulators of Cd (6000–130000 mg/kg dry weight) [4]. Some wetland plants can tolerate high concentrations of several metals in their tissues, which do not show negative effects on plant growth. Water zinnia (*Wedelia trilobata*) and smartweed (*Polygonum hydropiperoides*) accumulate 148 mg/kg^{-1} Cd in their shoot tissues without negative effects [5]. Many wetland plants accumulate higher concentrations of metals in roots than in shoots [6]-[10]. The greater accumulation of trace elements in roots was a common model in many studied plants [11]-[13].

Juncus acutus is shortly rhizomatous perennial plant and its height is more than 1.5 m [14]. It invades lowland grassland and grassy woodland, riparian vegetation, freshwater wetland (seasonal), and saline and subsaline wetlands [15]. *Juncus acutus* blooms in June. There are approximately 300 species of *Juncus acutus* and they are widely distributed throughout the world, occurring naturally in Africa, Europe and North America [16]. Also it is the most abundant plant in Kizilirmak grassland, Samsun, Turkey [17].

Amount of heavy metals in plants are started by metabolic requirements for minerals. However, some heavy metals such as Pb and Cd tend to be toxic to some species [18], [19]. Minerals in saline soils mostly accumulate in the roots, with small quantities move to the stems and leaves [20], [21]. The efficiency of *Juncus acutus* L. on the removal of heavy metals Cr, Ni, Cd and Zn, was investigated in a hydroponic experiment in order to evaluate its potential for use in the alternative remediation technology of constructed wetland systems [22].

No information available regarding the profile of heavy metals contents in stem and seed of *Juncus acutus* in Kızılırmak Delta. The objective of this study was to determine As, Cd, Sb, Ba, Hg and Pb concentrations of stem and seed of *Juncus acutus* and evaluate them for grazing animals and birds living in Kizilirmak Delta.

II. Material and Methods

A. Study area

The study area was on the Black Sea coast in the northern part of Turkey. The highest population of buffaloes present in Kızılırmak Delta where farmers leave their animals for 7

The authors thank to Ministry of Health, Public Health Laboratory for providing laboratory facilities for mineral analysis of samples.

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months starting from April to November throughout the year. Besides cattle, sheep and wild horses appear all the year around.

There are more than 352 bird species in Kızılırmak Delta, this is the highest value determined in an area in South Black Sea Basin. Kizilirmak Delta is a crucial wetland among others wetlands of Turkey especially for migratory birds in the world. Its location is internationally important because of being at bird migration routes in West Palearctic Region, passing through Turkey. Delta is the most important wetland for the migratory birds that pass over Black Sea; to rest after their long distance flight, to feed and to shelt [23].

B. Sample Collection

Seeds of *Juncus acutus* samples were collected randomly by hand, and stem samples were collected from 20 plants in July 2017, August 2017 and September 2017 from Kizilirmak Delta, Samsun, Turkey. The GPS coordinates of sampling locations were shown below.

- 36 19' 53 81" E, 41 16' 30 29" N
- 36 6' 51 52" E, 41 36' 24 24" N
- 36 5' 33 18" E, 41 38' 16 85" N

The stems were chopped into small pieces with garden scissors.

C. Experimental Procedure

Duplicate samples were prepared for each analysis. Samples were weighed and dried in an oven at 65 ºC. After drying, all stems and seeds were ground in a mill to pass through a 1 mm screen, and kept in plastic boxes until for mineral analysis. The concentrations of As, Cd, Sb, Ba, Hg and Pb were determined by an ICP-MS (Agilent–7700 Model) at the Public Health Laboratory, Ministry of Health, 55060 Samsun, Turkey.

0.5 g in 1 mm dimension stem and seed of *Juncus acutus* samples were put into vessels and the mixture composed of 10 mL of concentrated HNO_3, 3 mL of concentrated HCl and 2 mL H_2O_2 was added to each vessels. They were placed in Microwave Oven (Model CEM Mars) and were digested at two streps first 15 min at 180 ^oC then after secondly 30 min at 200 ^oC [24]. The resulting liquid samples were diluted with deionized water to bring the volume up to 50 mL for heavy metal ICP-MS analysis. Then the concentrations of As, Cd, Sb, Ba, Hg ve Pb were measured by ICP-MS method in prepared *Juncus acutus* samples [25].

D. Statistical Analysis

The obtained mean values of heavy metals As, Cd, Sb, Ba, Hg and Pb were subjected to ANOVA by one-way experimental design within the collected stem and seed samples of *Juncus acutus*. Duncan's multiple range test was used to compare the means [26]. Differences of means were considered significant at P<0.05.

III. Results and Discussion

The estimated mean values for As, Cd, Sb, Ba, Hg and Pb of stem are shown in Table I. The calculated mean values for As, Cd, Sb, Ba, Hg and Pb of seed of *Juncus acutus* are shown in Table II.

TABLE I
HEAVY METAL CONCENTRATIONS OF STEM OF *JUNCUS ACUTUS* (MEAN ± SE, N=20)

Month	July	August	September
Heavy metal (mg/kg)	($\bar{x} \pm S\bar{x}$) (n = 20)	($\bar{x} \pm S\bar{x}$) (n = 20)	($\bar{x} \pm S\bar{x}$) (n = 20)
As	0.11±0.03	0.12±0.01	0.10±0.03
Cd	0.07±0.04	0.05±0.02	0.05±0.03
Sb	0.02±0.02	0.01±0.02	0.03±0.01
Ba	2.26±1.01	2.33±0.21	2.34±0.25
Hg	ND	ND	ND
Pb	ND	ND	ND

ND: Not Detectable. SE: Standard error of the mean.

TABLE II
HEAVY METAL CONCENTRATIONS OF SEED OF *JUNCUS ACUTUS* (MEAN ± SE, N=20)

Month	July	August	September
Heavy metal (mg/kg)	($\bar{x} \pm S\bar{x}$) (n = 20)	($\bar{x} \pm S\bar{x}$) (n = 20)	($\bar{x} \pm S\bar{x}$) (n = 20)
As	0.10±0.09	0.10±0.05	0.09±0.04
Cd	0.06±0.03	0.06±0.02	0.07±0.03
Sb	0.02±0.03	0.02±0.01	0.02±0.03
Ba	2.05±0.85	1.92±0.12	1.88±0.22
Hg	ND	ND	ND
Pb	ND	ND	ND

ND: Not Detectable. SE: Standard error of the mean.

There were no statistically significant differences between monthly collections for both stems and seeds of *Juncus acutus*. Similarly there were not statistically significant differences for all the heavy metals between stem and seed of *Juncus acutus*. The concentrations of heavy metals were found detection limit in the stem and seed of *Juncus acutus* in three months.

The determined levels of As, Cd, Sb and Ba in stem and seed of *Juncus acutus* were very low. The concentrations of Hg and Pb were not detectable in both stem and seed of *Juncus acutus* during three months mainly July, August and September. Denise and Akhere [27] reported the mean concentrations of Hg for wetland plants mainly *A. zizanoides, T. mildbraedii* and *Cyclosporium. A. zizanoides* (0.330 mg/kg) had highest uptake potential for Hg while *T. mildbraedii* (0.030 mg/kg) had the least uptake potential for Hg. The uptake potential of wetland plants is dependent on species of plants. On the contrary, the mean concentration of Pb was determined 1.830 mg/kg in *T. mildbraedii*. This finding was attributed to corrosion of water pipes, industrial waste as well as domestic activities which were predominant in their study areas [28].

The Cd (0.07 mg/kg) and Sb (0.03 mg/kg) concentrations for stem of *Juncus acutus* were lower than that of *Juncus acutus* grown in Rio San Giorgia (1.04 mg/kg for Cd and 0.041 mg/kg for Sb) but the obtained concentration of stem for As was similar to reported value [28]. The As, Hg and Pb concentrations for stem of *Juncus acutus* were lower than those of *M spicatum* (0.11 mg/kg; 0.32 mg/kg, ND; 0.11 mg/kg, ND; 3.05 mg/kg) respectively [29]. The Pb (ND) and

Cd (0.07 mg/kg) concentrations of *Juncus acutus* were lower than those of *Myrophyllum spicatum* (10.81 mg/kg; 0.43 mg/kg) and *Batrachium aquatile* (25.16 mg/kg; 0.38 mg/kg) respectively [30]. Similarly, Singh et al. [31] reported that Cd and Pb concentrations of stem of *Panicum antidotale* were found 5.91 mg/kg and 75.42 mg/kg respectively. The As (0.32 mg/kg), Cd (0.07 mg/kg) and Pb (ND) concentrations of *Juncus acutus* were lower than those of *Juncus maritumus* (0.23 mg/kg, 0.97 mg/kg, 0.46 mg/kg), *Juncus effusus* (0.42 mg/kg, 1.79 mg/kg, 0.93 mg/kg) and *Juncus articulatus* (0.73 mg/kg, 1.54 mg/kg, 2.42 mg/kg) respectively [32]. The Ba (2.26 mg/kg) concentration for stem of *Juncus acutus* was similar to that of stem of *Phragmites australis* (2.20 mg/kg) [12]. The Pb concentration was not detectable in *Juncus acutus*. And this finding for Pb in *Juncus acutus* was similar to reported results for *Hydrocharis morsus-ranae, Nymphaea alba* and *Typha latifolia* by Sainty and Jacobs [14]. In another reported study, Cd (0.13 mg/kg) concentration of stem of *Juncus maritimus* was similar to that of *Juncus acutus* in our study [33]. While Pb concentrations of stem of *Juncus acutus* was not detectable, Pb concentrations of stem of *Juncus maritimus* were reported 1.01 mg/kg by Almeida et al. [33]. As and Cd concentrations of *Typha domingensis*, *Phragmites australis* and *Arundo donax* were < 0.1 mg/kg as those of *Juncus acutus* [34]. Bonanno et al. [35] reported that mean As concentrations in stems of *N. officinale* (0.10 mg/kg) and *A. Nodiflorum* (0.08 mg/kg) were in line with stem samples of *Juncus acutus* (0.11 mg/kg in July, 0.12 mg/kg in August, 0.10 mg/kg in September), however, Cd levels of *Juncus acutus* stem samples were lower than *N. officinale* and *A. Nodiflorum* plants. On the other hand Cd levels in the stem of *A. donax* were lower than stem of *Juncus acutus.* However, the reason why wetland plants would be innately tolerant to metals remains unclear. A possible explanation may be the biogeochemistry of the rhizosphere soils of wetland plants [36]. Moreover, element translocation from sediments to roots seems more influenced by the kind of plant species and trace element, whereas translocation across the various organs seems mainly species-specific. No clear patterns of trace element translocation were identified according to plant life form [37]. Distribution of the heavy metals in the different parts of plants appears in different concentrations. The accumulation of heavy metals takes place mostly in the root and slightly in the stem. The order of accumulation of heavy metals is as following: root > leaf > stem. However, for chromium the order was as root > stem > leaf. The level of heavy metal concentration varies with seasonal changes. Yabanli et al. [28] reported that the bioaccumulation of heavy metals in the plant was maximum in spring, because plant development was raised in spring and uptake also was enhanced. The order of heavy metals accumulation was As > Cr > Pb > Hg > Cd**.** Arsenic, one of the fatal toxic elements, is commonly found in the aquatic systems [38]. Li et al. [39] set out that As was determined in order as root tissue > root surface > stem tissue and the highest arsenic accumulation was found in the root. The highest arsenic was estimated as 15.30 mg/kg in the root and the lowest arsenic concentration was 0.32 mg/kg in the stem. Yucel et al. [40] studied Pb and Cd in *M. spicatum* in terms of indicator. Authors reported that the mean Pb values obtained from the stem and the leaves of the plant were 61.50 and 59.63 mg/kg respectively. The mean Cd levels of the stem and the leaves of the plant were 0.81 mg/kg and 1.81 mg/kg respectively. The obtained mean Pb and Cd values in stem samples of present study were quite lower than reported concentrations by Yucel et al. [40].

Almeida et al. [33] reported that the metal distribution such as Cd, Cr, Cu, Fe, Mn, Ni, Pb and Zn among the different tissues of *J. maritimus* changed according to the studied locations. These changes might result from many different factors such as sediment characteristics, size and age of plant and apparent physiological state.

Janadeleh et al. [41] reported that accumulation and toxicity of Cd in birds is more than plants in *T. australis*, Cu and Pb possess the highest translocation factor. Therefore, beside birds or other animals in the wetlands, the consumption of food obtained from grazing animals in wetlands may have health risk for human.

In conclusion, many other studies' result show that heavy metals were mostly found in roots and leaves of plant rather than stem. This is also in agreement with our findings. In this study, heavy metals were lower in stem and seed. Therefore, stem and seed of *Juncus acutus* are safe in respect to As, Cd, Sb, Ba, Pb and Hg for grazing animals and birds living in Kizilirmak Delta.

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