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COMPARATIVE STUDY OF PHYSIOLOGICAL AND PHENOTYPE CHARACTERISTICS OF JUNCUS RIGIDUS L. RHIZOME IN TWO REGIONS IN BASRAH GOVERNMENT

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Abstract
The work was conducted at the tow zones north and southern regions on Basrah city in the south of Iraq to investigate the impact of polluted elements Pb,Cd,and Ni in rhizome of *Juncus rigidus* L. species ,it found by the results the analysis of polluting elements Pb,Cd and Ni in the rhizome of *Juncus rigidus* species the level of Pb was higher value than the rest of the other polluting elements ,phenotype of rhizome appearances less thick and dark brown in color and dense thick and stiff bristles in the polluted zone, measurements such as length ,thick, hairs number also considered in dimention.RWC it recorded a reduction rate of 49.45% compared to the control .Phenols and flavonoids content in rhizome has enhanced significantly at $p \leq 0.05$ within polluted, the highest level was recorded in the southern region of basrah was 4.8mg/gm and 2.7mg/gm. phenols content in polluted zone and control respectively and flavonoids was 2.9 mg/gm and 1.6 mg/gm in the polluted and control zone respectively.

Keyword: Rhizome,phenotype,polluted elements,RWC,phenols and flavonoids,

Introduction

The *Juncus* from juncaceae family with spread all over the world if its found in Asia, Africa and south America (.Al-Obaidy,2016),genus Juncus Linnaeus,1753 approximately 250-300 species world wide. In flora of Iraq this genus has six subgenera (*Juncus*,*Genuini* ,*subulatia*,*Pseudotenageia*,*poiophylli* and *septati*).The Jenus Juncus commonly called rushes or samar.This genus is a perennial plant and all of its leaves are basal,terete,pungent auricles are absent .The flowers are in stalked panicles and seeds contain appendages (Townsend and Guest,1985).

The species Juncus rigidus Desfontaines ,1798 grown in marshes ,shallow brackish water,and semi-saline soil and can be found in moist soils,wet and temperate climates (snogerup,1978;Townsend and Guest,1985).

This species are used in traditional medicine because it contain antioxidant ,antimicrobial,antitumers cytotoxic,antiviral,antialgal and anti-flamnotory properties (El-shamy et al.,2015) ,Also contains several secondary metabolites sush as: Alkaloids,phenols and flavonoids which act as a natural antioxidants ,which act for removal substances agnist harmful free radicles (Al-Saadi,2013).The seeds of this plant rich with fatty acid and amino acids (Al-Saadi,2013).

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The most critically relevant environmental pollutants are heavy metals(Tangahu et al 2011).

The source of metals include natural rock erosion and human activities from industrial processes go into unpolluted areas where they accumulate in the water,soil.deep sediment, and living organisms (Miretzky et al ,2004) .

Some plants species have ability to remediate and absorb heavy metals (Lasat,2000) .The processes of phytoremediation can remove heavy metals such as : Fe,Mn,Zn,Cd,Cr,Pb,Co,Ag,So,Hg,Cu,Mg,Mo and Ni) (Najeeb et al .,2017).Plant species which collect for phytoremediation purposes is depend on plants being in open biological systems with latent to accumulate more heavy metal of dry biomass and growth rates

(Susarla et al.,2002;McGrath and Zhao,2003).Pollution can absorbed from soil particles or soil liquid via roots systems ,Also by cracks down of polluted sites from soils, sediment and water ,then go through translocation and bioaccumulation to the internal plant tissues (Cho-Ruk et al,2006;Paz-Al berto and Siqua,2013).

City of Basrah from Iraq contains developed industrial or urban regions which led to many environmental problems can caused increase in pollution, including heavy metals (Al-Obaidy et al.,2016).Many studies have evaluated and identified the sediment and water of the most pollution sites ,the heavy metals such as Pb,Cd and Ni are increasing in basrah city because its closeness to oil-Companies or industrial waste regions (Khwedim et al.,2009).The aim of this study was to determine the bioaccumulation of three heavy metals Pb,Cd and Ni in the Rhizome of J.rigidus in Tow regions the first grown in the south of and north of Barah city to determine the applicability of J.rigidus rhizome for phytoremediation and explain the phenotypic and physiological changes of the species-dependent rhizome .

Materials and Methods:

In the college of science the experiments were conducted in biology department,Basrah university. Three zones were selected ,zone 1 in the center of Basrah city(Control specimens) uncontaminated area Global positioning system GPS 47° 45° 46° E 30° 34° 46° N; zone 2 in the southern region from Basrah city(Al-Sha'eiba)Global positioning system 47° 41° 59° E 30° 22° 59° N and zone 3 in the north of Basrah city(Taga place)at Global positionning system 47° 43° 58° E 30° 39° 44° N.

The specimens of *J.rigidus* rhizome were collected in the summer of 2020 from the three zones and brought into the laboratory on the same day.removing the remaining soil from rhizomes ,then washed carefully three times with distilled then the measurements were taken ,and chemical experiment were conducted, then dried the specimens for chemical analysis to determents heavy metales(Ni,pb and Cd) in the rhizome of J.rigidus by HNO_3 digestion and filtrated mixture was subjected to an atomic spectrophotometer (Phoenix-986, CITY, England). Heavy metales in rhizome was determined through comparison to a standard curve (Kabata-Pendias and

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Pendias, 1992),Also the samples of soils were collected at 0 –15 cm depths,dried the soils in oven at 150 °C for 12 hours digested in acid-cleaned Teflon microwave vessels hydrofluoric acid2ml and 5ml of nitric acid and they were digested at 200◦C for 30 min (Binning and Baird, 2001),heavy metals were determined by using atomic spectrophotometer (Phoenix-986,City,England).the wave length of spectrophotometer as follow: Pb 217 nm; Cd -228.8 nm and Ni 232 nm and determent the concentration of heavy metals were 0.01ppm.

Evolution of phenols and Flavonoids:

The optical method with spectrophotometer were used to evaluate phenolic compound and a folin reagent ,the reaction mixture consisted of 1ml plant extract,9ml of distilled water,and1ml of folin reagent, then 7ml of 10%$NaCO_3$ WAS add, the absorbance was measured at 550nm and the phenolic compound were estimated in mg/gm unit ,according to the method of Milan,2011.

Flavonoids were evaluate according to method by Kaviarasan et al 2007 and Wei and Intan, 2008.The reaction mixture consisted of 1ml of plant extract and 4ml of distilled water, then 0.3ml of 5% $NaNO_3$,THE absorbance was read as 510nm and the flavonoid concentration was estimated as mg/gm. Yang,2018.

Statistical Analysis

One-way analysis of variance (ANOVA)were used to analyzed the data A significance level < 0.05 was considered statistically significant.

Table (1): Average concentrations of Lead, Nickel and Cadmium in Rhizome of *Juncus rigidus* (mg/kg^{-1}) .

Metal	Concentration		
	Zone1	zone 2	zone 3
Lead	3.55±2.46	15.55±2.46	10.32±2.11
Nickel	0.10±0.04	0.30±0.04	0.22±0.03
Cadmium	0.03±0.03	0.06±0.03	0.03±0.02

Concentration of heavy metal in *J. rigidus* rhizome tissue of contaminated zone are shown in table(1) the highest accumulation of the heavy metal was showed in lead 15.55±2.46mg/kg and then nickel 0.30±0.04mg/kg and the lowest value was recorded for cadmium 0.03±0.03mg/kg,these values showed the rhizome of *J.rigidus* can accumulated great amounts of heavy metales.This indicates that the element of lead exceeded the upper limits of the normal limit, that agreed with other literature on Dianthus carthuianorum was conducted by Baranowska&Wierzbika,2004 and other literature using other *juncus* species by Dweis and Weis,2004;Yanqun et al,2004;Deng et al,2004.We not by this result that the rhizome in the J.rigidus plant has can accumulate to heavy metals like root,stem,and the leaf as was noted in a previous study conducted by shatha et al.,2020 on the root ,stem and leaf of J. rigidus,as the results proved the accumulation

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of heavy metals in each of the root, stem and the leaf which the most part in which lead was accumulated.So agreed with Grube et al.,(2008) in the study on some species of Juncus are sensitive to heavy metal strees.The heavy metal specially lead very highly toxic pollutent cause reduced plant growth because a the binding of lead with the plasma membrane of cells, causing its control cell division and reducing tissue growth(Pourrt et al.,2011;Doncheva et al.,2013).
It was observed that the plant continued to survive despite the accumulation of heavy metals, in this case, the plant resorted to stimulating the activity of antioxidant enzymes that help remove this toxicity from cells,(Han et al.,2016).

Phenotype of rhizome:

The rhizome, which represents the transverse stem under the soil, carrying the roots down and the areal stem up, appeared to be variable in its appearance between the polluted and the control. The rhizome was less thick and dark brown in color and dense, thick and stiff bristles in the polluted area compared to the control area, which was thicker and brown in color lighter and fewer and more flexible bristles, figure (1) and this confirms with mentioned(Rodiyati&tupan.,2016).All parts of the plant are likely to be affected in appearance and compositing by the surrounding environmental conditions, especially the root hairs, as they are in direct contact with the soil, especially when it is polluted, causing changes in the size, length and hardness of these hairs, this was mentioned by(Anna et al.,2019) in a study on barely plant, where they noticed clear changes in the root hairs when changing the conditions surrounding caution and change in the level of nutrients when treated with other substances gold nanoparticles, He also mentioned by(Clanales et al.,2017) occurrence of clear changes in the root hairs when the surrounding environment conditions change, they noticed that these changes occur when the plant is exposed to a fluctuation in the level of nitrates, in other study conducted by(Burridge et al.,2019) on a group of plants before they mentioned the occurrence of clear flowering changes in the root hairs when these plants were exposed to stress conditions, figure (1) diagram (1).

A

B

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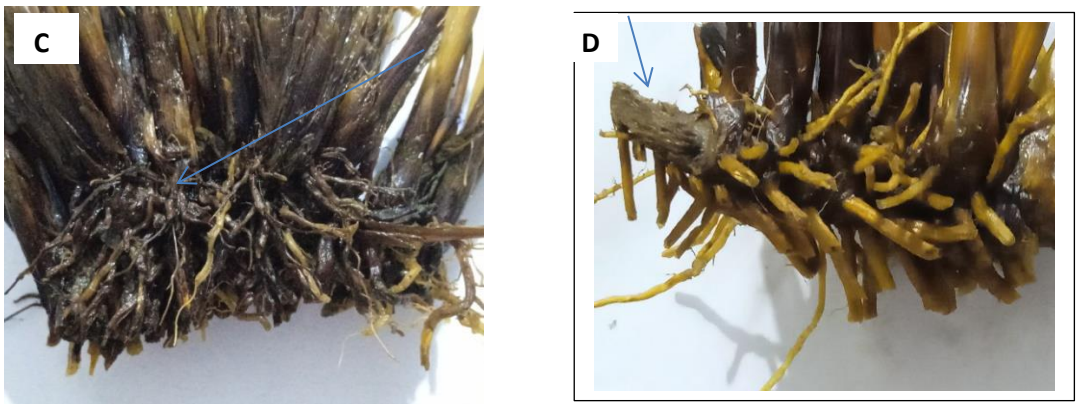
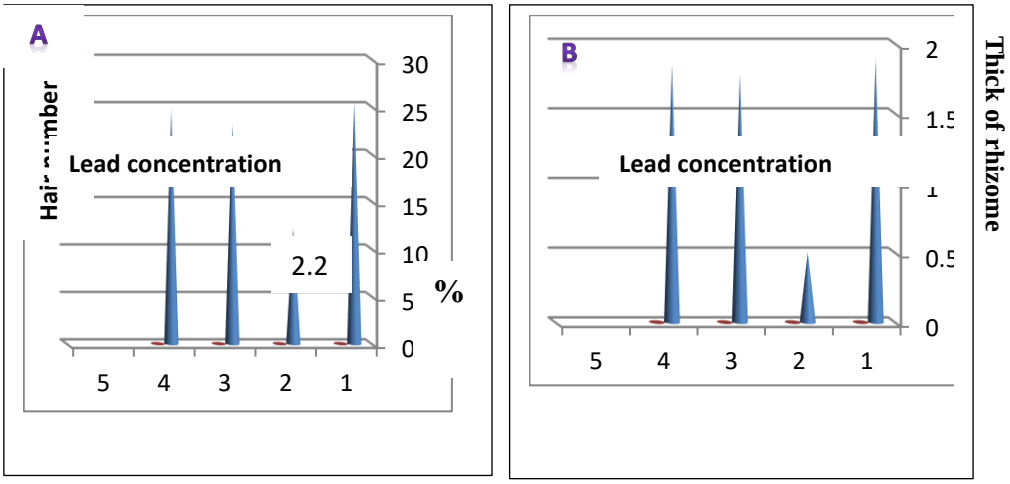


Figure (1)showed A rhizome of *J.rigidus* in polluted zone,B rhizome in control zone C rhizome hairs in polluted zone D,rhizome hairs in control zone.

Relative water content(RWC):

We not through the study conducted on the rhizome in the polluted zone compared to the control zone that the water content in the rhizome within the southern polluted Shaeiba zone was less than the control area. It recorded a reduction rate of 49.45% compared to the control area. As for the northern zone, the rhizome showed a little reduction with water content 11% compared to the control zone. The relative water content (RWC) is widely used to assess the state of water in the plant tissue under the influence of judgments (Munnse et al.,2006) and it is considered one of the important indicators for assessing the extent of plant tolerance of judgments (Agrawal et al.,2013) . diagram(2).



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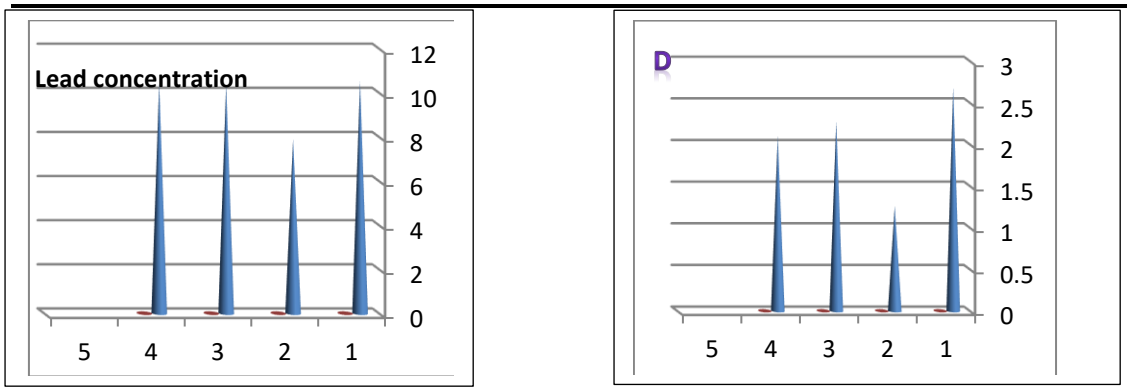
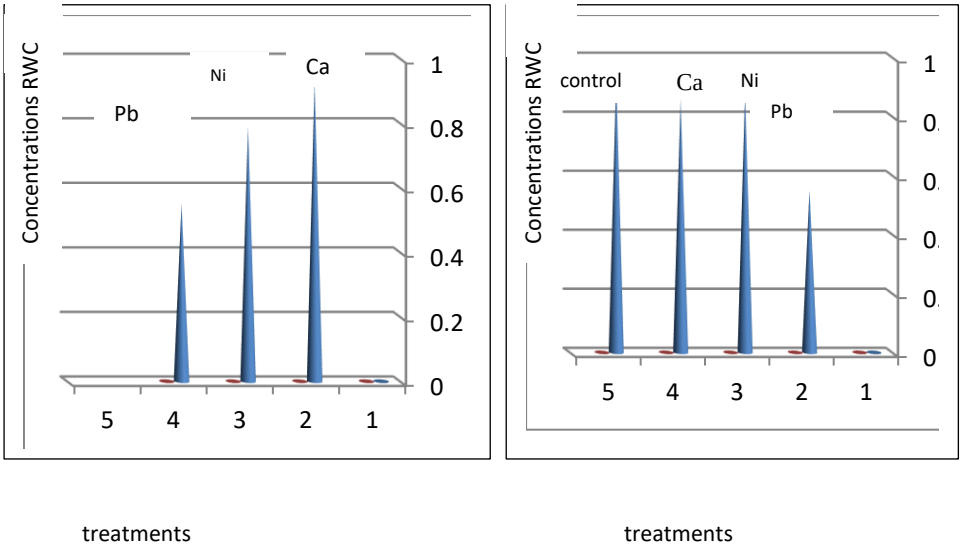


Diagram (1): Measurements of phenotype of rhizome of *Juncus. rigidus* in polluted zone compared with control,A:hiar number,B:thick of rhizome,C:length of rhizome,D:percent of hair number/length of rhizome. *J. rigidus* (in mg kg-1). P≤0.05**.**

We note in this study the level of contamination affected the water content in the rhizome, and this indicates that pb contamination affected the composition of the internal tissues of the rhizome and its gradation within the tissues that make up the rhizome. This phenomenon was also observed in other plants, it studied by (Charlotha&Rodiyati,2016) on sea grass Thalossia hemprichii,they mentioned that the polluting elements collect in the rhizome, as well as the roots and leaves. He mentioned that the pollutants affect the anatomy of the internal tissues of the rhizome, leading to a reducation in its composition, as well as due to the interaction of the polluting elements in the plant. Also mentioned(Raven et al.,2011) on Ranunculus sp. Due to the accumulation of polluting elements of the plant and enter ther[illegible]ith the plant nutrients.



Diagram(2) Relative water content with A:pb,Ni,Ca B:Compretaive effect of Pb,Ni,Ca with control p≤0.05.

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Phenols and Flavonoids content:

We note through the study and as shown in the diagram(3) that the content of phenols has enhanced significantly at p≤ 0.05 within the polluted zone. The highest level was recorded in the southern region of Basrah city, which is Al-Shaeiba region, compared to the northern region and the control region. The analysis of polluting elements,Ni,Ca,Pb it appeared that the level of pb was a higher value than the rest of the other polluting elements,therefore,an increase depending on the increase of pb. So, showed from the study that the polluted has significantly enhanced the level of phenols and flavonoids, Analysis of the rhizome, we found that pb pollution was more concentrated than elements, cadmium and nickel due to the proximity of the area to the oil companies and cars exhaust on the road through which it is clear that pb pollution one of the important factors in enhancing the content of phenolic and flavonoid substances in the rhizome, and this is consistent with what mentioned (Kaimoyo et al.,2008) ,they showed that contamination with heavy elements enhances the content of phenolic and flavonoid substances as a result of enhancing secondary compound in the plants when confronted with judgments. They also(Najafi and Jamei,2014) found that pb contamination enhances the synthesis of phenolic and flavonoid compounds, it was also(Alireza,2015) clarified through paper on the wheat plant exposed to contamination by pb the reason for the increase in the content of phenolic and flavonoid substances.

The stresses to which the plant is exposed are divers, including the contamination with heavy metales, so the phenolic and flavonoids compounds are considered to respond clearly to facing these stresses(Alizera,2015),phenolic substances act as resptores for metal chelates as well as that phenolic substances act with other compounds such as regulatory antioxidant enzymes in response to stresses by removing and scavenge molecular species of active oxygen (Kennedy et al., 2002).

Plants have many mechanisms for coping with changes. The environment during the growth period in which the plant tries to maintain the level of cellular balance and physiological processes necessary for (Yang et al.,2018;Amold et al.,2019) through primary metabolic compounds that change in plants when faced with judgments and secondary metabolites that play an important role in responding to environmental changes. Many researchers mentioned that the change that occurs in the secondary metabolic compounds is an important and main indicator that can be relied upon in evaluating the tolerance of plants to stress(Taiza and Zeiger ,2006).

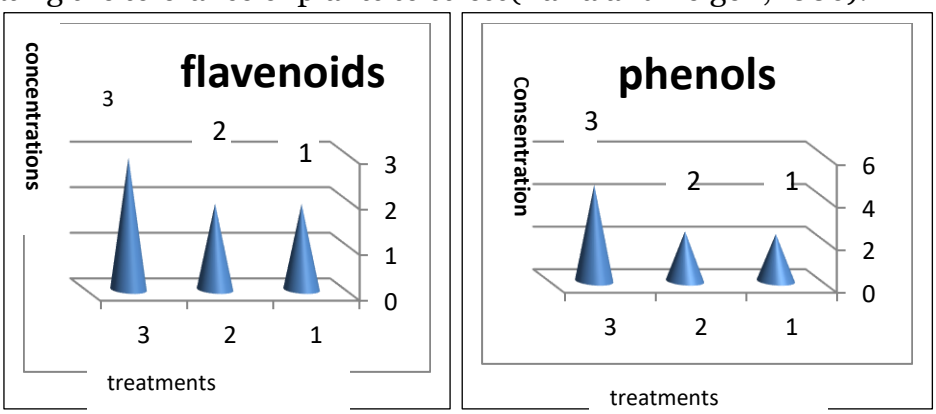

Diagram (3): Phenols and Flavonoids of rhizome of J.rigidus 1: control 2:northern zone 3: southern zone.

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Conclusion

By this study ,it could be recommended that rhizome of Juncus rigidus can accumulate polluted elements like other parts of plant ,pb wae highest value among other Cd and Ni ,So effect in diminution of rhizome and appearance,RWC in rhizome also affects by polluted elements comper with control and phenols and flavonoids content recorded the highest value in southern zone comper to control.

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