Risk evaluation of accumulated heavy metals for Radish (*Raphanus sativus* L. *var. sativus*) cultivars irrigated by varying water resources

Caser Ghaafar Abdel[1] **Aveen A Ibrahim[2]**
Professor **Lecturer**
[1] **Department of Field Crops / College of Agriculture /** University of AL-Muthanna
[2]**Chemistry Department / Science College/ Zakha University**
Email: caser.abdel@uod.ac

Abstract:

Three radish cultivars namely Ufasic, Gigante, and Saxa2 grown under plastic house, irrigated by familiar Life drinking water, well and Dohuk river wastewater to evaluate the risk of accumulated heavy metals in leaves and root of three radish cultivars. The obtained results manifested that a very high amount of Pb accumulate as compared to the international recommended standard. Maximum Pb limit for human health has been established for edible parts of crops (0.2 $mg.kg^{-1}$). Particularly, in edible leaves of radish irrigated with bottled water "life" and waste water (19.8641 and 15.6603$\mu g.g^{-1}$,respectively).Similar trends obsered in accumulated Pb in edible roots of life water (19.69$\mu g.g^{-1}$) and waster water (25.74$\mu g.g^{-1}$).Waste water significantly increased the accumulated Zn (9.732$\mu g.g^{-1}$), in edibile leaves of radish. Radish roots were not capable to sequestered Zn in their roots, and therefore, most absorbed Zn translocated to leaves. The lowst accumulated Cd in edible radish root (0.986$\mu g.g^{-1}$) observed in radish irrigated with well water. Saxa2 can be recommended for Pb bio remedy for extracting Pb from growing media. Ufasic and Saxa2 can be recommended for Zn extraction from growing media. Gigante engages the gap between them. Interaction results mentioned in results and discussion.

Key word: Pb, Cu, Zn, Cd, Heavy metal pollution, Health risk, wastewater, water resources, Radish ،

تقيم مخاطر تراكم المعادن الثقيلة في اصناف الفجل *Raphanus sativus L. var. sativus* المروية بمصادر مياه مختلفة

قيصر جعفر عبد [1] افين اكرم ابراهيم [2]

استاذ مدرس

[1]قسم المحاصيل الحقلية/ كية الزراعة/ جامعة المثنى

[2] قسم الكيمياء/ كلية العلوم / جامعة زاخو

البريد الالكتروني:caser.abdel@uod.ac

المستخلص :

نميت ثلاثة اصناف من الفجل (Ufasic, Gigante, and Saxa2) تحت البيت البلاستيكي ورويت بمياه نهر دهوك المخلوط بمياه المجاري وبمياه البئر وبمياه الشرب المنتج من شركة لايف لدراسة تراكم المعادن الثقيلة في الاوراق والجذور. حصل تركم عالي للرصاص في الفجل وتعدى الكمياة المسموح بها صحيا حسب المعايير العالمية (over 0.2 $mg.kg^{-1}$) خاصة في اوراق النباتات المروية بمياه الشرب لايف (19.8641 $\mu g.g^{-1}$) وبمياه نهر دهوك المخلوط بمياه المجاري (15.6603 $\mu g.g^{-1}$). وحصل نفس التراكم للرصاص في جذور الفجل المروي بمياه لايف (19.69$\mu g.g^{-1}$) وبمياه نهر دهوك المخلوط بالمجاري .(25.74$\mu g.g^{-1}$). ادى الري بمياه النهر الخلوط بالمجاري الى زيادة معنوية في محتوى الاوراق من الزنك (9.732 $\mu g.g^{-1}$). لم تتمكن النباتات من حجز المعادن الثقيلة بالجذور ومنع وصولها الى الاوراق وعلية حصل تراكم لمعظم الزنك في الاوراق. اقل تراكم للكادميوم كان في جذور الفجل المروي بمياه الابار (0.986$\mu g.g^{-1}$). صنف الفجل Saxa2 كان الاكفا في استخلاص الرصاص حيث يوصى باستخدامة في العلاج البايولجي للاوساط الملوثة بالرصاص ويوصى باستخدام صنفي Ufasic and Saxa2 في استخلاص الزنك من الاوساط الملوثة به.

Key word: Pb, Cu, Zn, Cd, Heavy metal pollution, Health risk, wastewater, water resources, Radish ،

Introduction:

The heavy metal resources in Iraq come from pesticides (Cu and Zn, As), car exhausted fuel and paints (Pb), and phosphate fertilizers (3,5) Heavy metals are not biodegradable, have long biological half-lives, and have the efficacies for accumulation in varying body parts, causing to harmful by effects (14, 23). Plants receive heavy metals deposit from air, contaminated soils and irrigation with contaminated water (7). Urban vegetable production is usually accompanied by animal and human health risk, particularly in Iraq countries where no strict attention for food contamination is paid (3,6). Farmers irrigate their farm by wastewater rich in harmful heavy metals like cadmium (Cd), chromium (Cr), copper (Cu), iron (Fe), nickel(Ni), manganese (Mn), lead (Pb) and zinc (Zn). Wastewater is also contaminated with biological agents such as pathogenic bacteria, coliform bacteria fungi, protozoans and nematodes. Irrigation with waste water poses serious health threats, such as a risk of bio magnifications of heavy metals and transmitting intestinal nematodes and bacterial infections especially to consumers and farm workers (21). The EC of the waste water recorded high value, since it associated with the presence of more ionic species in the soil (17). Pathak *et al*. (21) also reported higher EC (3.85 dS m^{-1}) of wastewater irrigated soil in Haridwar (Uttara hand), India. During the present investigation the contents of various cations Na^{+}, K^{+}, Ca^{+2} and Mg^{+2} and anions $PO4^{-3}$, $SO4^{2}$ was recorded significantly higher in the wastewater irrigated soil in comparison to bore well water irrigated soil and this might be due to the presence of higher values of these ions in the waste water (17) . Bhise *et al*. (8) reported that wastewater irrigation significantly increased the calcium, magnesium, potassium, and sodium in the agricultural soil. Kumer *et al*., (17) determined the accumulation of heavy metals in different vegetables namely carrot (*Daucus carota*), radish (*Raphanus sativus*), beet root (*Beta vulgaris*) and sweet potato (*Ipomoea batatas*) grown in municipal waste water irrigated soil in the vicinity of sewage treatment plant. They stated that wastewater was highly rich in plant nutrients and heavy metals. The waste water irrigation significantly increased the contents of heavy metals in the soil and vegetables grown in waste water, irrigated soil. Among different vegetables the maximum accumulation of Cd, Cr, Cu, Fe, Mn, Pb, and Zn were recorded in beetroot, and carrot (16). The heavy metal accumulation in vegetable sample and oral reference dose. They found that Values of Rf D for Cd (0.001mg.kg^{-1}.day^{-1}), Mn (0.041mg.kg^{-1}.day^{-1}), Cr (1.5 mg.kg^{-1}.day^{-1}), Co (0.043 mg.kg^{-1}.day^{-1}), Ni (0.02 mg.kg^{-1}.day^{-1}), Fe (0.70 mg.kg^{-1}.day^{-1}), Mo (0.009 mg.kg^{-1}.day^{-1}), Cu (0.04 mg.kg^{-1}. day^{-1}), and Zn (0.3 mg.kg^{-1}.day^{-1}). WHO (27) recorded the value of Rf D for Pb (0.0035 mg.kg^{-1}.day^{-1}). A value of health index more than 1 is not beneficial to human health. For adult residents, the average daily metal intake established to be 0.345 kg of vegetable, whereas average body weight considered as 60 kg (26). The objective of this investigation was to detect the Pb, Zn, Cu and Cd accumulations in leaves and roots three radish cultivars namely Ufasic, Gigante, and Saxa2 irrigated by well, waste water and bottled life drinking water as check. Our intention was to find which of these cultivars possesses the highest efficacy for absorbing and accumulating heavy metal in its biomass in order to utilize it in biological remedy of heavy metal. In contrast, radish cultivar that revealed the lowest accumulated heavy metals, which might be used for safe food productions.

Materials and Methods:

An experiment was conducted in the plastic house, Dohuk Research Center, Dohuk, Iraq , to investigate the responses of Ufasic, Gigante, and Saxa2 radish cultivars to varying irrigation water types namely bottled life water, Well water and Dohuk river contaminated with wastewater in terms of their growth and mineral nutritional values.Split within Complete Randomized Block Design (S-RCBD) was selected for this trail, where the main plot (A) represented by life bottled drinking water (a1), well water (a2) and wastewater contaminated Dohuk water (a3). While, sub main plot (B) was represented by Ufasic (b1), Gigante (b2) and Saxa2 (b3). Therefore, 9 treatments were included in this experiment, each was replicated 4 times.

Cultural Practices:

Trays of 74 cells filled with peat moss pressed to insure higher peat moss bulk density, and then arranged in three sets to match the main plot design. Two seeds were sown in each cell after they were brought up to field capacity, Seedlings were thinned to one plant per cell at the cotyledon leaves stage. Trays fertilized with mixed with irrigation water at rate 2.5g.l^{-1} Urea mixed with 2ml.l^{-1} foliar trace elements. This fertilizer mixture was applied on December, 18th , 31st , January 6th, 11th , 15th and 20th. At maturity plant were harvested, fresh leaves were weighed and then oven-dried at 55^{o}C for 73 hrs and then re weighted to record their dry mater and calculating their dry matter percentages.

Mineral analysis:

Dry leaves were powdered and then samples of 0.5g were digested with 10 cm^{3} and perchloric acid exposed to 240C^{o}, and then diluted with 50cm^{3} deionized water. Lead, Copper, Zinc, and Cadmium measured by Absorption Spectrophotometer (GBC 932 AA, Avanta Ver 1.32).

Results and Discussions:

1. Mineral accumulations

A. Influence of applied water types

Accumulated Pb in dry and edible leaves (table, R1; figure, R1-2) revealed that the highest Pb accumulation in edible leaves and leaf dry matter (19.8641 and 148.033µg.g^{-1}, respectively) confined to radish irrigated by drinking life water, which significantly exceeded radish irrigated with well water and waste water. The lowest accumulated Pb in edible leaves (15.6603µg.g^{-1}) accompanied to waste water and to well water (119.35µg.g^{-1}) detected in leaf dry matter. Insignificant differences detected in the accumulated Pb in dry matter of radish roots (table, R2 ; Figure, R3-4) among different applied water types. However, the accumulated

Pb in edible roots of radish irrigated with well water was substantially lower than life and waste water watering types (12.168µg.g^{-1}). Life irrigated radish showed the highest Pb accumulation in root and leaf dry mattes followed by waste water and then well (figure, R15). The same trend manifested in edible root and leaf tissues, where radish irrigated with wastewater and life exhibited the highest accumulated Pb in root and leaf edible tissues. The lowest accumulated Pb recorded in radish irrigated by well water (figure, R16). The obtained results suggested very high amount of Pb accumulate as compared to the international recommended standard. Maximum Pb limit for human health has been established for edible parts of crops (0.2 mg.kg^{-1}). (10). WHO (28) recorded the value of Rf D for Pb (0.0035 mg.kg^{-1}.day^{-1}).The highest accumulated Cu in leaf dry matter and leaf edible tissue and confined to life water (15.53 and 2.0576 µg.g^{-1}, respectively), as they substantially exceeded these of radish irrigated by well and waste water (table, R1; figure, R5-6),the lowest accumulated Cu observed with radish irrigated by waste water. Insignificant results (table, R2; figure, R7-8) detected among varying applied water in the accumulation of Cu in root dry matter,however, well water significantly reduced the accumulated Cu in edible root tissue, where life and waste water manifested the highest values (25.76 and 32.05 µg.g mg.kg^{-1}.day^{-1}, respectively). Radish grown by life showed the highest Cu accumulation in plant dry matter followed by radish irrigated by wastewater, while the lowest observed in radish (figure, R17). Radish irrigated with wastewater manifested the highest Cu accumulation in edible leaf and roots followed by radish irrigated with life, while the lowest recorded in radish watered with well water (figure, R18). These results suggested profoundly higher accumulation of Cu in root, as compared to leaves. Similar results were reported by Abdel and Yousif (5). Cu accumulation in roots not in leaves were extremes when compared to WHO (2.63 to 3.36 mg.kg^{-1}). The critical food Cu threshold for human health has been established to be 10 mg.kg^{-1} (11) .

Waste water significantly increased the accumulated Zn (72.675 and 9.732µg.g^{-1}, respectively), in dry leaves and edible leaves of radish (table, R1; figure, R9-10). However, insignificant differences observed between life and well water types in both leaf dry matter and leaf edible tissues. The lowest accumulated Zn in edible radish root (0.07361µg.g^{-1}) observed in radish irrigated with well water (table, R2; figure, R11-12). However, insignificant differences detected among other watering types. These results suggested that radish roots were not capable to sequestered Zn in their roots, and therefore, most absorbed Zn translocated to leaves. The highest Zn in plant dry matters and plant edible tissues detected in radish irrigated with waste water followed by life and then well water (figure, R19-20).

The lowest accumulated Cd in edible radish root (0.986µg.g^{-1}) observed in radish irrigated with well water (table, R2; figure, R13-14) , which was substantially lower than that accumulated in radish roots irrigated by life (2.15µg.g^{-1}) and waste water (2.539µg.g^{-1}) . However, insignificant differences detected among other watering types. These results disagreed with that obtained in lettuce (3) and radish (5). They found that radish and lettuce irrigated by well water accumulated higher Cd than lettuce and radish irrigated by waste water. Theses contradictory obviously attributed to varying well water resource.) The FAO/WHO (13) limits for the heavy metal intake based on human weight for a range of adult of 60 kg body weight. The average diets for daily person consumption of vegetables and fruits are 98 and 78 g, respectively. If the mean levels of Pb (0.473 mg.kg^{-1}),Cd (0.071 mg.kg^{-1}), Cu (2.63 mg.kg^{-1}), Zn (3.7mg.kg^{-1}), Co (0.58mg.kg^{-1}), and Ni (1.49 7 mg.kg^{-1}) found here are consumed daily, the contribution of heavy metal intake for an average human being from the fruit diet is 36.89 µg, 5.54 µg, 0.205 mg, 0.288 mg, 45.24 µg, and 0.116 mg, respectively. In case of vegetables, if the consumed daily mean levels of Pb, Cd, Zn, Cu, Co, and Ni are 0.25, 0.14, 8.15, 3.36, 0.51 and 0.24 mg.kg^{-1}, respectively, the corresponding estimated daily intake will be 24.8 µg, 13.3 µg, 0.8mg, 0.33 mg, 49.7µg, and 0.0231 mg, respectively (12).

Table (R1): Accumulation of heavy metals (µg.g^{-1}) in leaves, as irrigated by varying water types.

Treat.	Dry matter of leaves			Edible tissue of leaves		
	PB	Cu	Zn	Pb	Cu	Zn
Life	148.033A	15.35A	64.408B	19.8641A	2.0576A	8.6717B
Well	119.35C	13.6833B	61.967B	17.3896B	1.8034B	8.1283B
Waste	129.925B	10.725C	72.675A	15.6603C	1.4237C	9.732A
Figures of shared characters' insignificantly differ, at 0.05% Duncan test						

Table (R2): Accumulation of heavy metals in radish roots edible and dried tissues, as irrigated by varying water types (µg.g^{-1})

T reat.	Dry roots				Edible roots			
	Pb	Cu	Zn	Cd	Pb	Cu	Zn	Cd
Life	94.12A	120.88A	0.6417A	10.125A	19.69A	25.76A	0.135AB	2.15A
well	88A	99.8A	0.525A	7.683A	12.168B	13.99B	0.07361B	0.986B
Waste	99.03A	118.14A	0.6917A	8.808A	25.74A	32.05A	0.19318A	2.539A
Figures of shared characters insignificantly differ at 0.05% level, Duncan test								

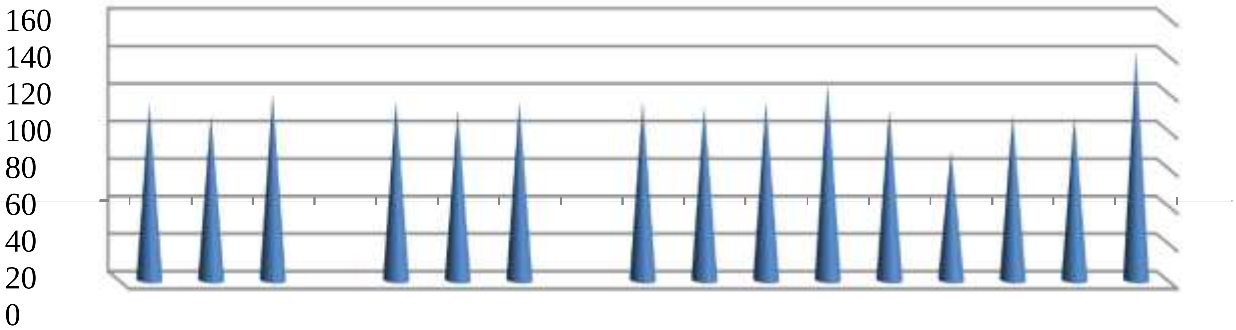

Figure (R1): Accumulation of Pb (µg per g dry matter) in leaves of radish cultivars irrigated by life, well and waste water.

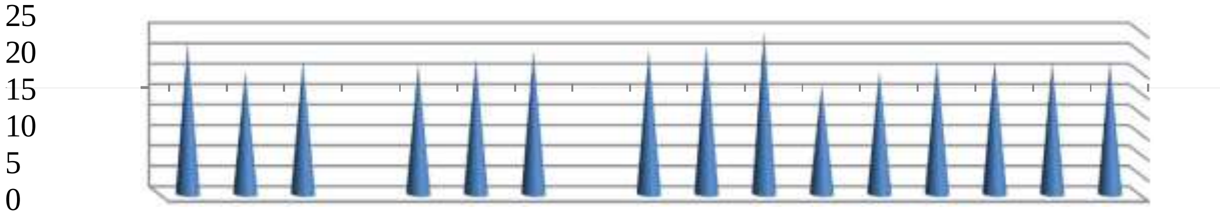

Figure (R2): Accumulation of Pb (µg per g edible leaves) of radish cultivars irrigated by life, tab and waste wate.

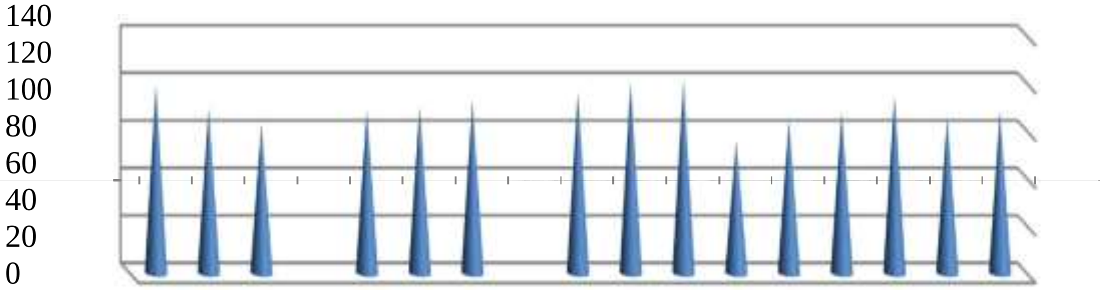

Figure (R3): Accumulation of Pb (µg per g) in dry roots of radish cultivars irrigated by life, well and waste water.

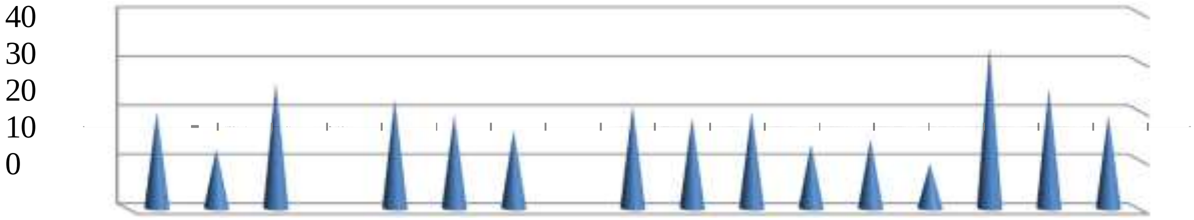

Figure (R4): Accumulation of Pb (µg per g) in edible roots of radish cultivars irrigated by life, well and waste water.

B. Cultivar responses to heavy metal accumulations.

The highest accumulated Pb in leaf dry matter (139.917 µg.g^{-1}), and edible leaves. (18.1256 µg.g^{-1}) observed in Saxa2 radish cultivar, which substantially exceeded other cultivars (table, R1; figure, R1-2). On the other hand, insignificant difference found among investigated cultivars in term of Pb accumulation in dry end edible roots (table, 2; figure, R3-4). Saxa2 showed the highest tendency for Pb accumulation in dry matter of root and leaves followed by Gigante and then

Ufasic (figure, R15). Ufasic showed the highest tendency for Pb accumulation in edible roots and leaves followed by Saxa2 and the lowest accumulated Pb recorded with Gigante (figure, R16) . These results suggested that Saxa2 cultivar manifested higher capability in accumulating Pb in its tissues, as compared to other cultivars. Therefore, Saxa2 recommended for Pb bio remedy for extracting Pb from growing media. The interaction amongst the varieties and treatments significantly differed for various parameters. Thus, it can be concluded, that the use of sewage water and Pb contaminated wastewater results in higher metal concentration in the radish root and may lead to different types of health problems to consumers. The Pb uptake by the root and leaf of radish plants increased by the increasing the applied Pb levels, with the highest value for root (19.008 mg kg^{-1}) and leaf (16.134 mg kg^{-1}) in the treatment receiving the highest applied Pb concentrations (15). The highest accumulated Cu (30.316µg.g µ$g.g^{-1}$) in edible roots of Ufasic. However, insignificant differences detected among other treatments (table, R4; figure, R8). Ufasic cultivar showed the highest accumulated Cu in leaf and root dry matters followed by Gigante and then the lowest recorded in Saxa2 (figure, R17). Once more Ufasic showed the highest tendency for Cu accumulation in leaf and root edible tissues followed by Gigante and then Saxa2 (figure, R18). These results suggested that Ufasic cultivar was suitable for utilization in bioremedy for substrate contaminated with Cu. Yang *et al*. (30) studied the response of three vegetables to Cu toxicity and found that Cu levels in both root and shoot increased, but root Cu concentration increased more sharply than shoot with increasing Cu levels in growth media. In relation to consumer health, it was found that soil total and available Cu thresholds for potential dietary toxicity in the edible parts of vegetable crops were 5-fold higher than those for phytotoxicity (at 10% yield reduction). Among the three vegetable crops, pakchoi had much lower soil total and available Cu thresholds, as compared with the other two vegetable species (24). Data also showed that the intake of most of the metals constitutes less than the TMDI (theoretical maximum daily intake) at present and hence health risk is minimal. However, with increase in vegetable consumption by the community the situation could worsen in the future. Treatment of industrial effluents and phyto - extraction of excess metals from polluted environments could reduce health risk (9). Moreover, consumers are supplied with well-performed acquired systematic resistance, which enable the intestine to select the desired ions. Therefore, consuming foods of high fiber contents is perfect for adsorbing the repelled harmful ion with disposed stool. The highest accumulated in edible leaf (9.3946µ$g.g^{-1}$) confined to Ufasic radish cultivar (table, R3; figure, 10).Similar results of accumulated Zn in dry roots (0.7833µ$g.g^{-1}$) and edible roots (0.19581µ$g.g^{-1}$)

accompanied to Ufasic radish cultivar, which highly exceeded Gigante cultivar. However, substantial differenced of Ufasic and Saxa2 were not detected (table R4;figure,11-12). These results suggested that Ufasic and Saxa2 cultivars showed higher capabilities in accumulation Zn in their tissues, particularly in roots, which confirm the abilities of these cultivar to sequestered Zn in roots rather than translocate it to leaves. Subsequently, Ufasic and Saxa2 can be recommended for Zn extraction from growing media. Ufasic and Gigante showed very close values of Zn accumulations in plant dry matters and plant edible tissues, while Saxa2 showed the lowest accumulation of Zn (figure, R19-20). Differences among species and even among cultivars in the absorption mineral ions of were recorded in radish (4, 5), in cauliflower and Broccoli (4), in lettuce (2, 19). Zn level of 25 $mg.l^{-1}$ shoot Zn concentration of Chinese cabbage was almost 2-fold lower than that of pakchoi or celery Zinc concentration in the edible part of celery was nearly 2-fold higher than that of the other two species when grown at higher Zn levels ($50mg.l^{-1}$). Moreover, under soil, the zinc accumulation coefficient (AF) in shoots increased for pakchoi, but decreased for celery and Chinese cabbage when soil available Zn was raised from 18 to $172mg.l^{-1}$.However, root Zn AF increased to varied extents, with increasing soil Zn for all the vegetables. Celery showed the highest AF in edible parts at low soil Zn i.e. CK (control), whereas pakchoi had the highest AF of Zn at higher soil available Zn levels. The AF for zinc in edible parts of the three vegetable crops decreased in the order: pakchoi, celery (stem) and Chinese cabbage. Significant positive correlations noted between shoot Zn and soil available Zn level (18).

Table (R3):Cultivar responses in accumulation of heavy metals in leaves, as irrigated by varying water types ($\mu g.g^{-1}$)

Treat.	Dry matter of leaves $\mu g.g^{-1}$			Edible tissue of leaves		
	Pb	Cu	Zn	Pb	Cu	Zn
Ufasic	126.025B	1.6882A	68.808A	17.2451A	1.6882A	9.3946A
Gigante	131.367B	1.766A	67.15A	17.5434A	1.766A	8.9647AB
Saxa2	139.917A	1.8306A	63.092A	18.1256A	1.8306A	8.1727B
Figures of shared characters' insignificantly differ, at 0.05% Duncan test						

Table (4): Cultivar responses to accumulation of heavy metals in radish roots edible and dried tissues, as irrigated by varying water types ($\mu g.g^{-1}$)

Cultivars	Dry roots				Edible roots			
	Pb	Cu	Zn	Cd	Pb	Cu	Zn	Cd

Ufasic	95.808A	120.4A	0.7833A	7.863A	22.322A	30.316A	0.19581A	1.9717A
Gigante	90.325A	109.9A	0.3667B	10.533A	19.032A	23.225AB	0.08664B	2.2594A
Sexa2	95.071A	108.28A	0.7083A	8.4A	15.973A	18.254B	0.11938AB	1.444A
Figures of shared characters insignificantly differ at 0.05% level, Duncan test								

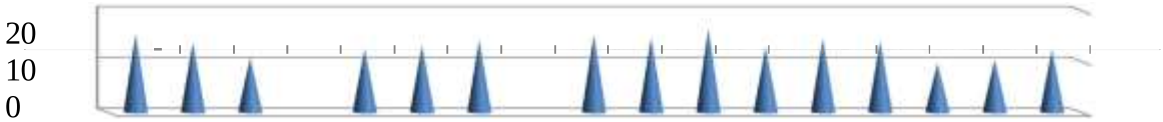

Figure (R5):Accumulation of Cu (µg per g dry matter) in leaves of radish cultivars irrigated by life, well and waste water

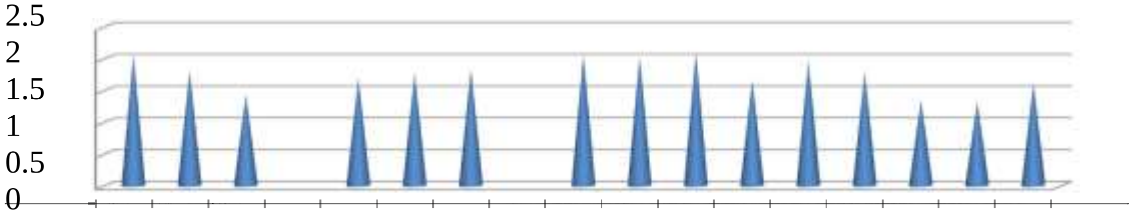

Figure (R6): Accumulation of Cu (µg per g edible leaves) of radish cultivars irrigated by life, well and waste water

C. Cultivar responses to varying water types

The highest accumulated Pb in dry matter of leaves (158.825µg.g^{-1}) and edible leaves (20.366µg.g^{-1}) observed in Saxa2 cultivar irrigated with life water. Wile, the lowest dry leaves Pb (106.725 µg.g^{-1}), and edible roots Pb (13.914 µg.g^{-1}) detected in Ufasic cultivar irrigated with well water (table, R5; figure, R1-2). The obtained results suggested the water types effects dominated the cultivar responses, where the worst accumulation confined to life water. On the other hand Pb accumulation in dry roots and edible roots overwhelmed by wastewater where the highest Pb accumulation found in dry root (121.6 µg.g^{-1}), accompanied by Sax2 irrigated with wastewater and in edible roots (32.397 µg.g^{-1}), of Ufasic cultivar irrigated with wastewater (table, R6; figure, 3-5). However, the lowest Pb accumulation in dry roots (68.7 µg.g^{-1}) and in edible roots (9.217 µg.g^{-1}), fond in Saxa2 cultivar irrigated with well water. Saxa2 radish cultivar irrigated by life and wastewater showed the highest accumulated Pb in root and leaf tissues (figure, R15). The highest accumulated Pb in edible leaf and root tissues found in Ufasic radish irrigated by wastewater, while the lowest Pb accumulation Saxa2 irrigated by well water (figure, R16). These results confirmed higher Pb accumulation in leaves than that accumulated in roots, which disagreed with the previous results reported by (5,6), which revealed that roots sequestered Pb and inhibited its translocation to leaves. This contradictory attributed to the earlier harvesting of roots before they attained their final size. The total biomass of radish cultivars were insignificantly influenced the applied Pb concentrations and sewage water, except for root diameter, which was significantly greater in the local cultivar (3.261 cm). Pb treatments significantly reduced the growth and yield of both the cultivars. The total biomass, fresh weight of root and root diameter was significantly higher, except for Pb 400 $mg.l^{-1}$, in the plants receiving sewage water as compared to the control and different levels of Pb (15). It was reported that when seedlings of two rice (*Oryza sativa* L.) cultivars were raised in sand cultures under 500 and 1000 µM Pb(NO3)2 in the medium, lengths as well as weights of roots and shoots decreased with increase in Pb concentration. Thus, Pb readily absorbed by growing seedlings, its localization was greater in roots than shoots (25). The maximum permissible Pb concentration in waste water that was agreed on is 84 $mg.Kg^{-1}$ soil

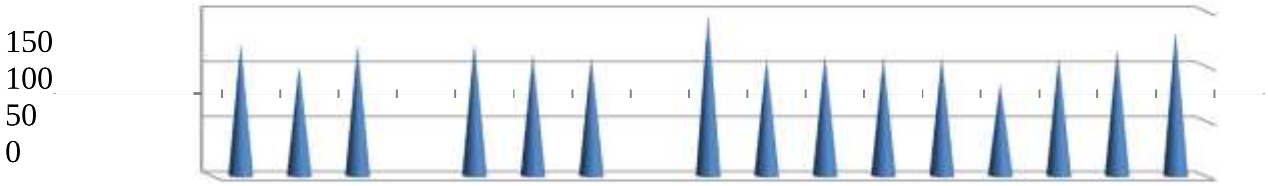

Figure (R7): Accumulation of Cu (µg per g) in dry roots of radish cultivars irrigated by life, well and waste water.

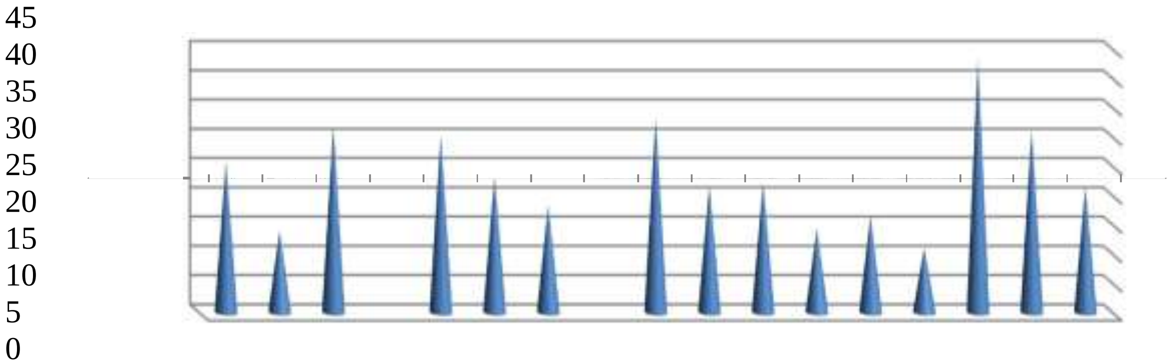

Figure (R8): Accumulation of Cu (µg per g edible roots of radish cultivars irrigated by life, well and waste water

The highest accumulated Cu in dry leaves (16.35$µg.g^{-1}$) and in edible leaves (2.091$µg.g^{-1}$)
confined to Saxa2 radish cultivar irrigated with life water. However, the lowest Cu accumulation in dry leaves (9.6 $µg.g^{-1}$) detected in Ufasic cultivar irrigated by wastewater, and the lowest edible leaves accumulation of Cu (1.3159 $µg.g^{-1}$) observed in gigante cultivar irrigated by wastewater (table R5; figure, 5-6). The highest Cu accumulation in dry roots (146.08$µg.g^{-1}$), confined to Ufasic irrigated by life water and in edible roots (43.331$µg.g^{-1}$), found in Ufasic cultivar irrigated by wastewater. While, the lowest accumulated Cu in dry roots (83.8$µg.g^{-1}$), and

edible roots (11.066µg.g^{-1}) found in Saxa2 irrigated with well water (table, R6; figure, R7-8). Ufasic irrigated with life revealed the highest accumulated Cu in their leaf and root dry matters, followed by Saxa2 irrigated by wastewater. However, the lowest accumulated Cu in Saxa2 irrigated by well water (figure, R17). The result confirmed that Ufasic irrigated by wastewater and life possesses the highest tendency for Cu accumulation in their edible root and leaves, followed by waste water irrigated Gigante cultivar. However, the lowest tendency for Cu accumulation recorded in Saxa2 irrigated by well water (figure, R18). These results manifested that Ufasic cultivar showed higher capability in detaining Cu in roots, as compared to other cultivars by inhibiting its translocation to leaves regardless to irrigation water types. Moreover, Ufasic cultivar appeared to have a higher tendency for heavy metal accumulations, particularly in roots. Resemble results reported by Ejazul *et al*., (24) who stated that Cu mainly accumulated in roots while a small fraction (10%~20%) of absorbed Cu was transported to shoot. Celery accumulated higher Cu content both in roots (1557 mg.l^{-1}) and shoot (166.7 mg.l^{-1} in leaves). Yang *et al*. (30) reported that from the regression lines between shoot DM yields and Cu concentration in plant tissue or soil, soil Cu thresholds for phytotoxicity (10% yield reduction) and potential dietary toxicity in edible parts of the vegetables could be calculated. Mineral concentrations ranged from 0.02 to 1.824, 0.75 to 6.21, 0.042 to 11.4, 0.141 to 1.168, 0.19 to 5.143, and 0.01 to 0.362 mg/kg for Pb, Cu, Zn, Co, Ni, and Cd, respectively. However, the highest levels of Pb, Cu, Zn, Co, Ni and Cd were detected in mango, melon, spinach, banana, mango, and mango fruits, respectively (13). The highest accumulated Zn in dry matter observed in all cultivars (72.05 to 73.15µg.g^{-1})irrigated with wastewater. While the lowest (56.925µg.g^{-1}), observed in Saxa2 watered with well water. The highest accumulated Zn in edible leaves observed in Ufasic irrigated by waste water (10.3856µg.g^{-1}). While the lowest was (7.3208µg.g^{-1}) confined to Saxa2 watered with well water (table, R5; figure, R9-10). The highest Zn accumulation in dry roots and edible roots (0.9 and 0.3127 µg.g^{-1}) accompanied to Ufasic cultivar irrigated by wastewater. However, the lowest accumulated Zn (0.225 µg.g^{-1}), in dry roots and edible roots (0.044 µg.g^{-1}) found in Gigante cultivar irrigated with well water (table, R6; figure, R10-11). All cultivars irrigated with wastewater showed the highest accumulated Zn in plant dry matters and plant edible tissue followed by Gigante irrigated with life and Ufasic irrigated by well (figure, R19-20). Data showed that Zn concentration in Celery, Mint, Dill, Spinach and Green pepper were more than Zn permitted, where the critical dietary Zn threshold for human health has been established to be 20 mg.kg^{-1} (11). The maximum

Zn tolerance for human health has been established for edible parts of crops (20 mg.kg^{-1}). Zn threshold for human health has been established to be 20 mg/kg (11). Ejazul *et al*., (24) found that soil available Zn thresholds for Zn potential dietary toxicity were 175.6, 74.9 and 101.0 mg/kg for Chinese cabbage, pakchoi, and celery (stem), respectively. For pakchoi, a higher soil available Zn threshold for yield reduction (10%) (103 mg/kg) was again noted relating to that for potential dietary toxicity (74.9 mg.kg^{-1}).The highest Cd accumulation in edible roots (3.248µg.g^{-1}) confined to Gigante cultivar irrigated by wastewater, and the lowest (0.681 µg.g^{-1}) coincided with Ufasic cultivar irrigated with well water (table, R6; figure, R14). Insignificant differences detected in accumulated Cd among all dual interaction treatment. Similar variations between vegetable cultivars observed in previous investigations. Ni *et al* (28) Studied Chinese cabbage (*Brassica chinensis* L. cv. Zao-Shu 5), winter greens (*B. rosularis* var. Tsen et Lee cv. Shang-Hai-Qing), and celery (*Apiumg graveolens* L. var. dulce DC). They found that the cadmium concentrations in shoots and roots varied both with different Cd levels and type of vegetable. Cd accumulation in various plant parts in vegetable crops increased with the increasing cadmium concentrations in the growth medium. Root Cd increased more sharply than shoot Cd. Celery contained higher Cd in the edible parts than other vegetable species. The maximum permissible Pb concentration in waste water that was agreed on is 4mg.Kg^{-1}soil. The lead (Pb) concentration in all vegetable samples was more than maximum permitted concentrations, while Cd pollution was observed in radish, Cress, Dill, spinach and eggplant (24). Data showed that metal uptake differences by the vegetables are attributed to plant differences in tolerance to heavy metals and vegetable species (9). Accumulation mechanism of heavy metals in plants manifested higher contents of metals like Cd, Cu, Fe, Mn and Zn in their tissues, and only a small amount of these metals is stored in the roots and the rest will be translocated to their aerial parts. Therefore, wastewater irrigation significantly increased the content of Cd, Cr, Cu, Fe, Mn, Pb and Zn in the carrot, radish, beet root and sweet potato (20, 17).

Table (R5): Accumulation of heavy metals in leaves, as irrigated by varying water types (μg.g^{-1})

Treat.	Dry matter of leaves			Edible tissue of leaves		
	Pb	Cu	Zn	Pb	Cu	Zn
Life Ufasic	140.25BC	15.125AB	65.95AB	19.079AB	2.0584AB	8.9649AB
Life Gigante	145.025B	14.58ABC	68.075AB	20.148A	2.0234AB	9.4683AB
Life Saxa2	158.825A	16.35A	59.2B	20.366A	2.091A	7.5819B
Well Ufasic	106.725E	12.75BC	67.65AB	13.914C	1.664ABC	8.8333AB
Well Gigante	120.025D	14.4ABC	61.325AB	16.085CB	1.9585AB	8.2309B
Well Saxa2	131.3DC	13.9ABC	56.925B	16.983B	1.788ABC	7.3208C
Waste W Ufasic	131.1DC	9.6E	72.825A	18.743AB	1.342C	10.3856A
Waste W Gigan-	129.05DC	10.325DE	72.05A	16.398CB	1.3159D	9.195AB
Waste W Saxa2	129.63DC	12.25DC	73.15A	17.028B	1.6132BC	9.6155AB
Figures of shared characters' insignificantly differ, at 0.05% Duncan test						

Table (R6): Accumulation of heavy metals in three radish cultivars roots edible and dried tissues, as irrigated by varying water types (μg.g^{-1})

Treatment	Dry roots				Edible roots			
	Pb	Cu	Zn	Cd	Pb	Cu	Zn	Cd
Life Ufasic	94.85AB	146.08A	0.825A	11.825A	20.922ABC	33.286AB	0.18704AB	2.5467CAB
Life Gigante	92.75AB	106.48AB	0.225 B	10.625A	18.464BC	21.755CB	0.04434 B	2.0843CAB
Life Saxa2	94.75AB	109.5AB	0.875A	7.925A	19.678ABC	22.227CB	0.17371AB	1.8193CAB
well Ufasic	104.4AB	107.65AB	0.625AB	5.05A	13.106BC	14.331CB	0.08768 B	0.6817C
well Gigante	90.9AB	107.95AB	0.325AB	10.575A	14.18BC	16.58CB	0.04829 B	1.2658C B
well Saxa2	68.7 B	83.8B	0.625AB	7.425A	9.217C	11.066C	0.08486 B	1.0101C B
Waste Ufasic	88.18AB	107.48AB	0.9A	6.175A	32.937A	43.331A	0.3127A	2.6866 AB
Waste Gigante	87.33AB	115.4AB	0.55AB	10.4A	24.452AB	31.34AB	0.16729AB	3.428A
Waste Saxa2	121.6A	131.55AB	0.625AB	9.85A	19.025ABC	21.469CB	0.09956 B	1.5026C B
Figures of shared characters insignificantly differ at 0.05% level, Duncan test								

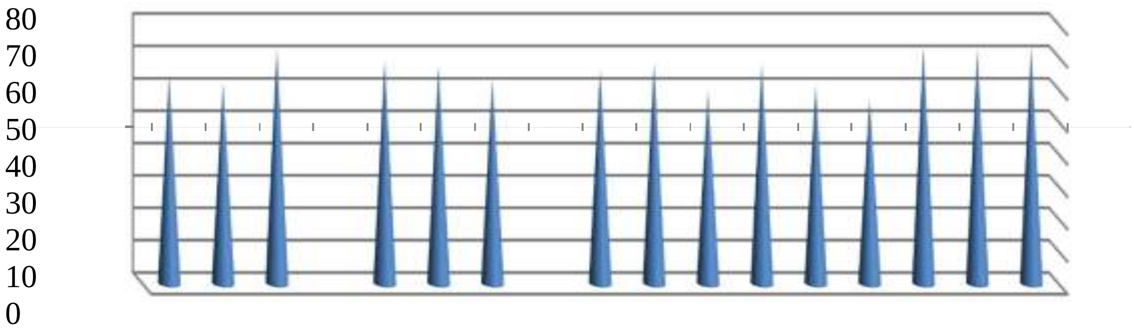

Figure (R9): Accumulation of Zn (μg per g dry mater) in leaves of radish cultivars irrigated by life, well and wastewater

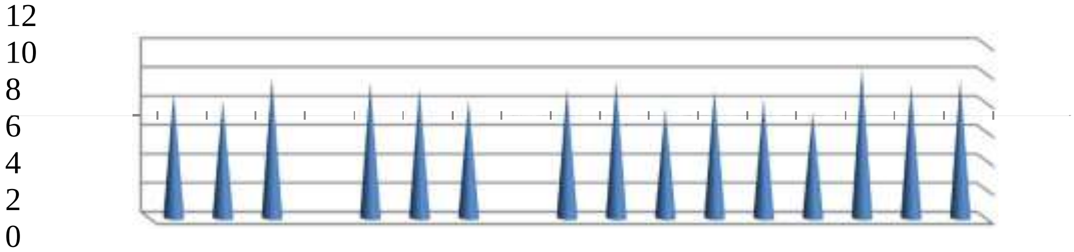

Figure (R10): Accumulation of Zn (µg per g edible leaves) of radish cultivars irrigated by life, well and waste water.

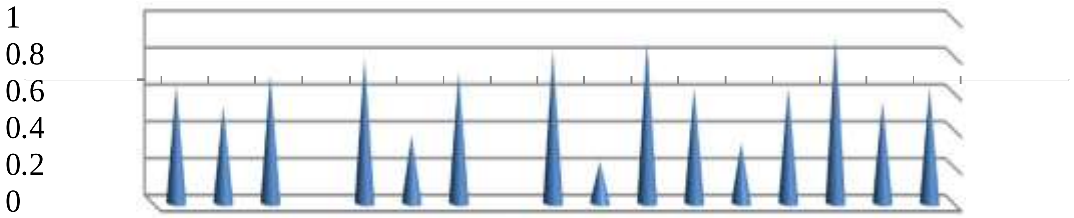

Figure (R11). Accumulation of Zn (µg per g) in dry roots of radish cultivars irrigated by life, well and waste water

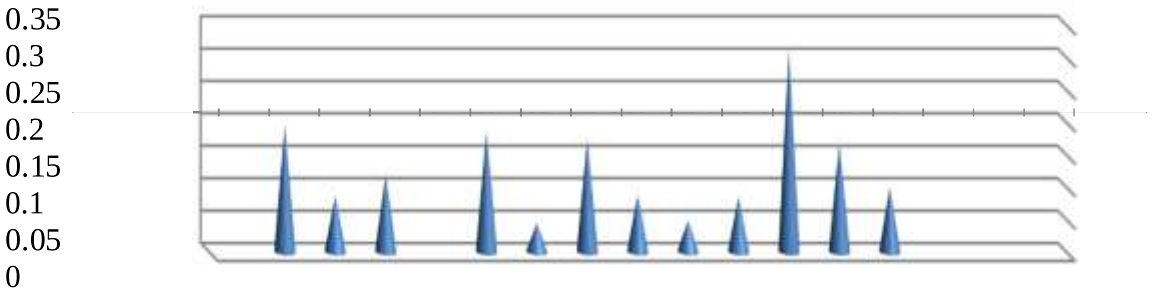

Figure (R12): Accumulation of Zn (µg per g edible roots) in leaves of radish cultivars irrigated by life, well and wastewater

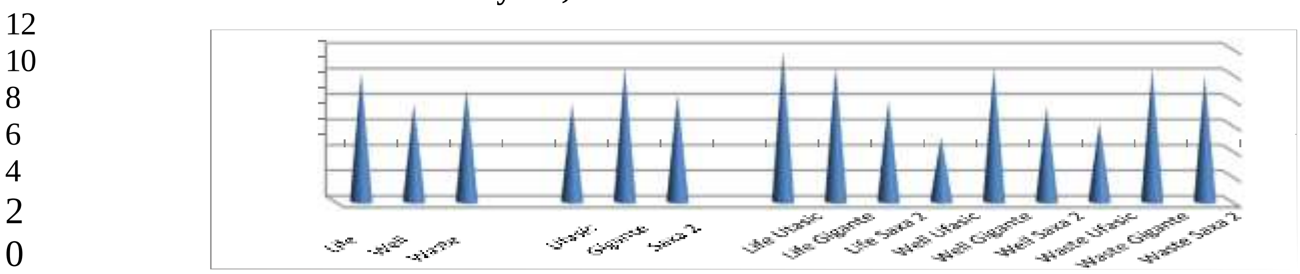

Figure (R13):Accumulation of Cd (µg per g) in dry roots of radish cultivars irrigated by life, well and waste water.

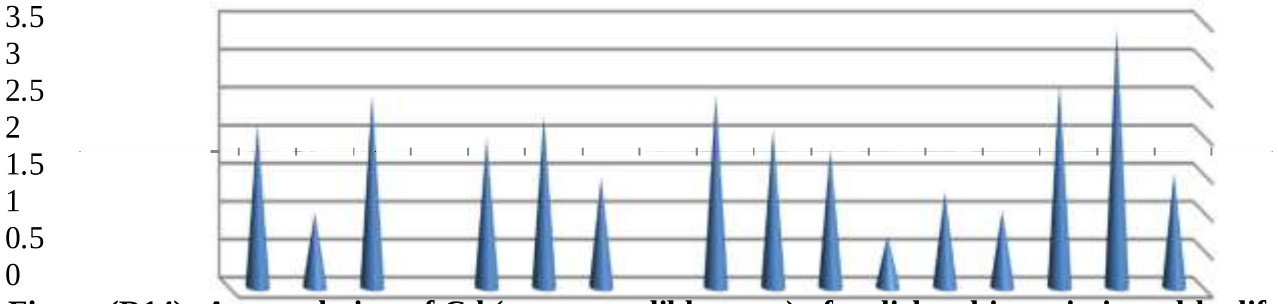

Figure (R14): Accumulation of Cd (µg per g edible roots) of radish cultivars irrigated by life, well and wastewater

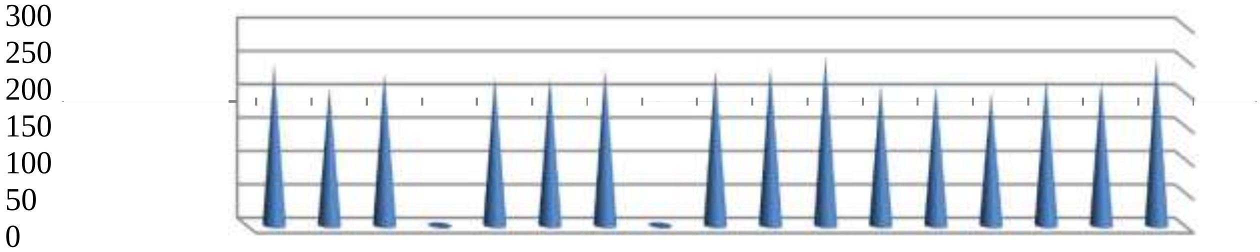

Figure (R15):Accumulation of Pb (µg per g) in dry leaf and root of radish cultivars irrigated by life, well and wastewater

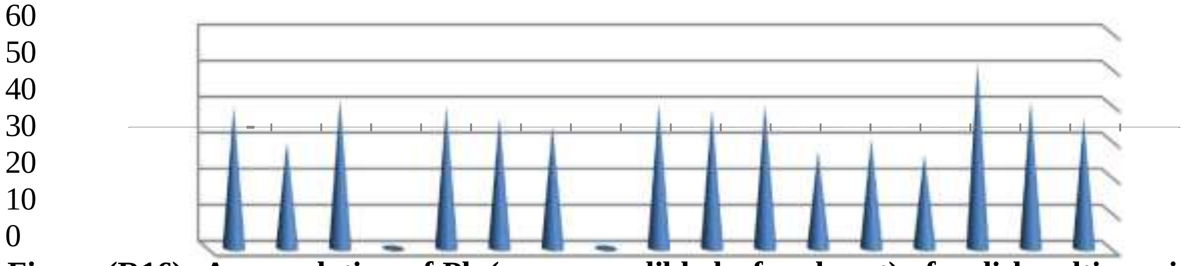

Figure (R16): Accumulation of Pb (µg per g edible leaf and root) of radish cultivars irrigated by life, well and waste water.

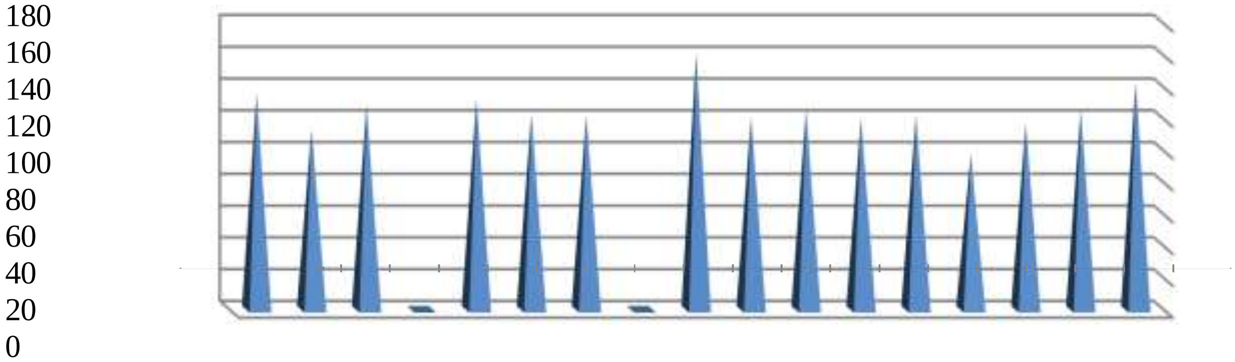

Figure (R17): Accumulation of Cu (µg per g) in dry matter of leaves and roots of radish cultivars irrigated by life, tab and waste water.

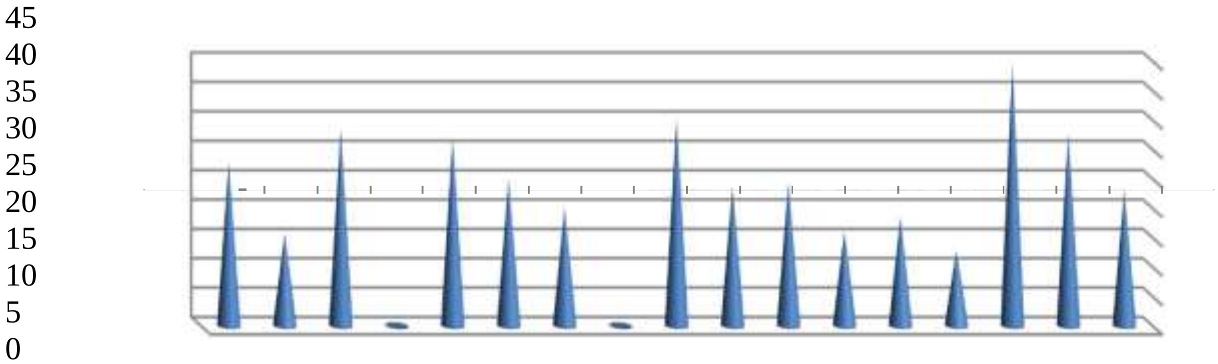

Figure (R18): Accumulation of Cu (µg per g) in edible leaves and roots of radish cultivars irrigated by life, tab and waste water.

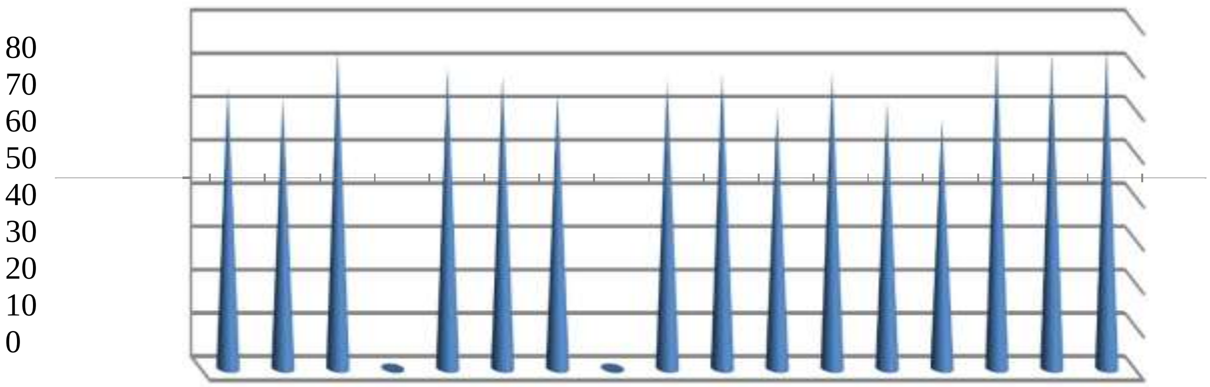

Figure (R19): Accumulation of Zn (µg per g) in dry leaf and root of radish cultivars irrigated by life, well and waste water.

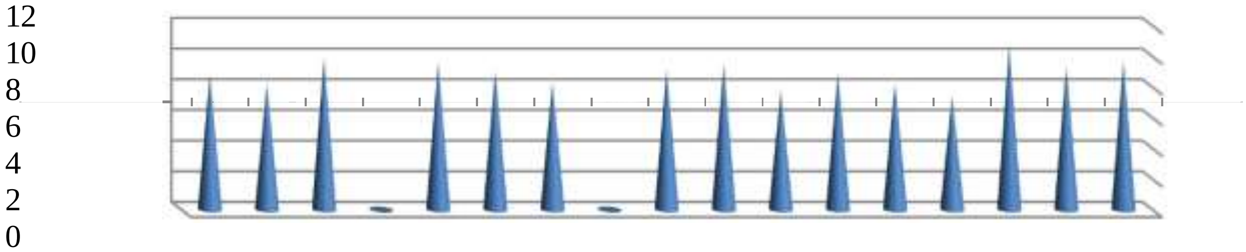

Figure (R20): Accumulation of Zn (µg per g) in edible leaf and root of radish cultivars irrigated by life, well and waste water.

2. Growth responses

The best performance of radish fresh and dry weights observed with irrigated by well water (figure, R21-22). Ufasic was the most responded cultivar followed by Gigante and then comes Saxa2. The highest plant fresh, and dry weight observed in Ufasic Irrigated with well water, and the lowest occurred in Gigante irrigated by waste water. Heavy metals risks attributed to the negative effects of them on the plant cell organelles, particularly on cellular membranes (3). It was found that seedlings grown for 5- 20 days in presence of 1000 µM Pb (NO3)2 showed about 21- 177% increase in the level of thiobarbituric acid reacting substances (TBARS) in shoots indicating enhanced lipid peroxidation compared to controls. With increase in the level of Pb treatment in situ peroxidases showed more increase in activity than SOD. Under Pb treated and untreated plants, roots maintained higher activity of peroxidases enzymes than shoots. Pb treated seedlings showed elevated levels of lipid peroxides with a concomitant increase in the activities of the enzymes superoxide dismutase (SOD), guaiacol peroxidase, ascorbate peroxidase and glutathione reductase compared to controls. It was found that about 87- 100% increase in SOD activity, 1.2- 5.6 times increase in guaiacol peroxidase activity and 1.2- 1.9 times increase in ascorbate peroxidase activity was observed in the roots of seedlings grown for 15 days in presence of 1000 µM Pb in the medium. Under similar treatment conditions about 128- 196% increase in glutathione reductase activity was recorded in roots and 69-196% increase in shoots compared to control grown seedlings. Pb treatment resulted in a decline in catalase activity in roots whereas in shoots catalase activity incr eased in seedlings grown at moderately toxic Pb (500 μM) level whereas a highly toxic Pb (1000 μM) level led to a marked inhibition in enzyme activity. Two catalase isoforms were detected in roots and three in shoots of the seedlings. A highly toxic Pb (1000 μM) level led to decrease in the intensity of two preexisting catalase isoforms in shoots. Results suggest that Pb induces oxidative stress in growing rice plants and that SOD, peroxidases and Glutathione reductase (GR) could serve as important components of antioxidative defense mechanism against Pb induced oxidative injury in rice (25). Shoot fresh weight (FW) progressively decreased with increasing Cu levels in the nutrient solution. Great differences in Cu tolerance were also noted among the three vegetable crops. Shoot fresh weight of pakchoi, celery and Chinese cabbage decreased to about 33%, 37% and 50% of the control, respectively, when grown with Cu supply of 10 mg/L. It was found that celery is more tolerant to the toxicity of Cu than Chinese cabbage or pakchoi grown in nutrient solution (30). Xiong and Wang (29) showed significant adverse influence of Cu on seed germination of *Brassica pekinensis*. The 0.5 $mmol.l^{-1}$ Cu treatment remarkably reduced the germination rate, and the *LC*50, calculated as the lethal effect on seed germination, was 0.348$mmol.l^{-1}$. Root and shoot, lengths of the young seedlings inhibited by Cu, however, stimulatory elongation of the shoots occurred with the 0.008$mmol.l^{-1}$ treatment. No visible Zn toxicity symptoms observed in the soil experiment, shoot growth significantly inhibited at Zn levels above 200 mg/kg for celery and Chinese cabbage, and above 300 mg/kg for pakchoi. Shoot dry weight decreased by 10% for Chinese cabbage and celery, but increased by 13% for pakchoi when grown at the soil diethylenetriamene pentaacetic acid (DTPA) extractable Zn level of 72 mg/kg. However, at soil DTPA-Zn levels up to 172 $mg.kg^{-1}$, similar yield reduction was observed with celery and pakchoi (18). Root DW reduced more than shoot DW for pakchoi grown at high Zn levels. Sensitivity of root and shoot growth to Zn toxicity was noted for celery. However, that pakchoi required higher soil Zn concentrations for optimal growth and was more tolerant to Zn at soil available Zn concentrations less than 132 mg/kg than the other two vegetable species (18). Ejazul *et al.*, (24) found that the total soil Zn thresholds for shoot dry matter yield reduction were higher for pakchoi and only slightly lower for celery (stem), than that for potential dietary toxicity.

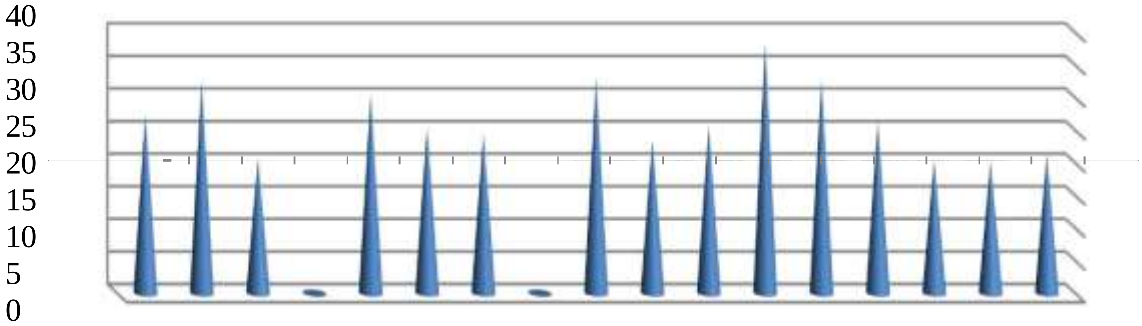

Figure (R21):Plant Freeh weight (g) of radish cultivars irrigated by life,well and waste water.

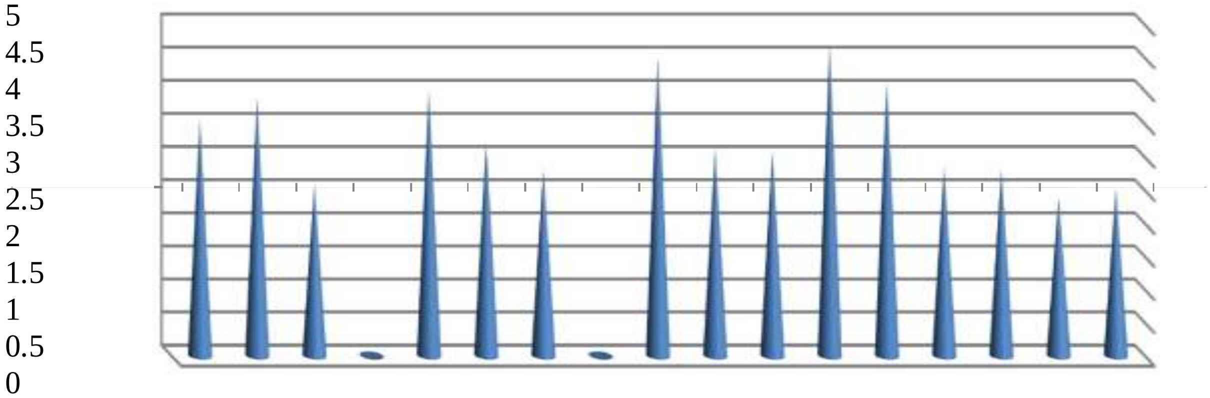

Figure (R22): Plant dry weight (g) of radish cultivars irrigated by life, well and waste water

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