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HYPER ACCUMULATION OF HEAVY METALS BY CRUCIFERAECEA VEGETABLES OF SRILANKA

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Abstract

As in most of Asian countries leaf vegetables are the main vegetables in Sri Lanka. Cultivation of crops in or close to contaminated sites may result in both growth inhibition and tissue accumulation of heavy metals. Some Leafy vegetables have the huge ability to absorb heavy metals and accumulate in plant parts. Therefore, this research was conducted to give detailed information regarding heavy metal accumulation in Crucifereacea vegetables in Sri Lanka and to emphasize the output for heavy metal contamination and samples were tested for Cu, Cr, Zn and Pb accumulation of five leafy vegetables species of family Cruciferaceae namely Brassica oleracea (cabbage), Brassica oleracea(Kohlrabi), Brassica oleracea (cauli flower), Brassica oleracea (collard green) and Raphanus sativus (Raddish). All the samples were collected from market site of Welimada, Dambulla and Jaffna districts. Samples were prepared using dry ashing technique and concentrations of heavy metals were found using Atomic absorption spectrometer. The results revealed that, the estimated concentration of the lead of selected vegetable varieties are greater than the WHO limit for lead intake, the consumption of average amount of these contaminated vegetables may cause health risk for the consumers. The estimated concentration of Cu, Zn and Cr of selected vegetable varieties can be suggested that the consumption of average amount of these contaminated vegetables do not pose a health risk for the consumers as the values obtained are below the WHO limits. This study concludes and the results of statistical analysis show, that heavy metal concentration varied among the test vegetables as well as areas and the consumption of average amounts of these contaminated vegetables poses a health risk for the consumers.

Keywords: Cruceferaecea vegetable; Hyper accumulation; Heavy metals

1. Introduction

A heavy metal is any relatively dense metal or metalloid that is noted for its potential toxicity, especially in environmental contexts. The presence of toxic metals in soil has severely inhibited the biodegradation of organic contaminants (Monuarora et al., 2008). Hyper accumulation of heavy metal in plants is defined as capable of growing in soils with very high concentrations of metals, absorbs these metals through their roots, and is concentrated extremely high levels of metals in their tissues. Metal hyper accumulator plant species are able to accumulate at least 0.1% of the leaf dry weight in a heavy metal (Hashmi et al., 2005).

Heavy metals have constituted an ill-defined group of inorganic chemical hazards. Heavy metal contamination has pose risk and hazards to humans and the ecosystem through direct ingestion or contact with contaminated soil, the food chain (soil-plant-human or soil-plant- animal-human), drinking of contaminated ground water and reduction in food quality via phytotoxicity (Alan et al., 2002). It may also affect the quality of agricultural soils, including phytotoxicity and transfer of heavy metals to the human diet from the crop up take. Heavy metals cannot be destroyed but can only be transformed from one oxidation state or organic complex to another (Chove, 2006). Remediation, vertification, immobilizations activities reduce the heavy metal range (Bandara, 2003). Generally, humans are exposed to these metals by ingestion (drinking or eating) or inhalation (breathing). These metals and their compounds increases ones' risk of exposure, Subsistence lifestyles can also impose higher risks of exposure and health (Duruibe, 2007).

Hyper accumulated plants hold interest for their ability to extract metals from the soils of contaminated sites (phytoremediation) to return the ecosystem to a less toxic state. The plants also hold potential to be used to mine metals from soils with very high concentration (phytomining) by grew the plants then harvested them for the metals in their tissues. The ability of brassicas to bio accumulate heavy metals can be used to reduce the level of contamination in the soil (phyto remediation) and thus to clean up and prepare soils for cultivation (Doherty et al., 2012). Cruciferacea plant can accumulate high amount of toxic metals, without visible symptoms and this has to be taken in to account in phyto remediation process (Rumeza, 2006).

Even though it has essential nutrients, it also has toxic compounds over a wide range of concentrations. Heavy metals present in these vegetables cause a wide range of human health hazards, from short-term impacts such as headaches and nausea to chronic impacts like cancer, reproductive harm, and endocrine disruption.

The pesticides contain many heavy metals. The uses of pesticides also participate in the heavy metal contamination. Children are particularly affected to the hazards associated with pesticides. Use of pesticide can damage agricultural land by harming beneficial insect species, soil microorganisms, and worms which naturally limit pest populations and maintain soil health.

Agriculture is synonymous with Sri Lanka due to the country's fertility and a varied agro climatic area as well as our country is rich in vegetation. Therefore, Sri Lankans have also consumed high amount of vegetables in daily life. But they don't consider the heavy metal accumulation due to the consumption of vegetables. In Sri Lanka accumulation of heavy metals in soil, water and plant/animal biomass has been widely reported. Heavy metals are rich in Cruciferaceae like plants. Vegetables plants grew on heavy metal contaminated medium can accumulate high concentration of trace elements to cause serious health risk to consumers.

Some researchers have reported about the estimation of heavy metals present in vegetables cultivated in different areas of Sri Lanka. Accumulation of heavy metals in soil, water and plant/animal biomass has been widely reported in Sri Lanka. In Sri Lanka and other regions in South Asia, the heavy metal and organic pollutant contamination already pose a severe threat to human and ecosystem health.

The main objective of this study was to assess the present status of trace metals pollution in vegetables produced from Jaffna, Dambulla and Welimada and to analyse the level of Pb, Cu, Zn and Cr concentration in Crucifereacea vegetables as well as to investigate possible hyper accumulation of heavy metals by cruciferaecea vegetables in Sri Lanka from the cultivated site (study area) in Jaffna, Dambulla and Welimada.

2. Materials and methods

Jaffna, Welimada and Dambulla cities were selected for the study. In these three sites, farming activities were carried out throughout the year but with domestic and industrial wastewaters being used to treat the soils during dry seasons. Cruciferous vegetable samples were collected from the market of these areas. The metals of interest included are copper (Cu), lead (Pb), Zinc (Zn) and Chromium (Cr). The results obtained from this study were useful for assessing the metals contamination as well as determining the need for remediation. The results had also provided information for background levels of metals in the vegetables in the study area.

2.1 Sample collection

The vegetables were analyzed including the edible portion for the five cruciferous vegetable varieties Brassica oleracea (Cabbage), Brassica oleracea (Kohlrabi), Brassica oleracea (Collard greens), Brassica oleracea (Cauliflower) and Raphanus sativas (Radish).

Samples were collected over a period of two months during the initial stage of research. Five samples of each vegetable were collected from each area, making a total of fifteen samples of each vegetable at the end of two-month period. Each sample was collected randomly on cultivated area market sites and wrapped in a big brown envelope and labeled and immediately brought to the laboratory for analysis.

2.2 Sample preparation and analysis

2.2.1 Pre- treatment

In the laboratory, each sample was washed with tap water and thereafter with distilled water to remove the surface pollutants. Then the stalks were removed from the leafy green vegetable portion. Samples were cut into small pieces and allowed to dry on paper about 2 hour to eliminate the excess moisture. Then it was dried in an oven at 80°C for 24 hours.

At the end of the drying, the oven was turned off and left overnight to enable the sample to cool to room temperature. Each sample was grounded into a fine powder; it was sieved by 2mm sieve. Finally it was stored in clean dry air tight glass containers at room temperature.

Fig 1. Grounding of vegetable sample

2.2.2 Dry ashing technique

A dry ashing technique was followed by an atomic absorption spectrophotometric analysis. One gram of the ground sample was measured and taken into clean crucible. Then crucible with sample was placed in a muffle furnace and ashed at 350oC for 12 hours.

Fig 2. Part of sample prepared for AAS analysis

Then the ash was digested with 5 ml of 20 % (v/v) HCL solution. The residue was filtered into a volumetric flask using a filter paper and the solution was made to the 50ml marked with deionized water.

2.3 Instrumentation

An atomic absorption spectrophotometer was used to analyze the samples and the results were compared with WHO standards. All stock standard solution of lead, Chromium, Zinc and copper was prepared as per instruction manual of atomic absorption spectrophotometer.

2.4 Statistical analysis

Non-parametric Kruskal – wallis statistical analysis was performed to analyze the summarized data obtained for concentration of different tested metals for different vegetable varieties.

For all possible pairs, the hypotheses to be tested are;

H0: There is no significant difference between pairs
H1: There is significant difference between pairs

Decision Rule: if p value < α value, then reject H0

3. Results and discussion

Table 1 indicates the pb, Zn, Cr and Cu concentrations of five cruciferaecea vegetables collected from four randomly selected markets in Jaffna, Welimada and Dambulla area.

Table 1. Quantify of heavy metal found in vegetable produced in different area

Pairs	Number	Concentration(ppm)			
		Lead	Copper	Zinc	Chromium
Jaffna-Cabbage	**1**	**0.124**	**0.176**	**0.225**	**0.234**
Jaffna-Radish	**2**	**0.163**	**0.171**	**0.524**	**0.251**
Jaffna-Kohlrabi	**3**	**0.199**	**0.783**	**0.365**	**0.345**
Jaffna-Cauliflower	**4**	**0.162**	**0.912**	**0.654**	**0.358**
Jaffna-Collard greens	**5**	**0.153**	**0.995**	**0.555**	**0.399**
Dambulla-Cabbage	**6**	**0.204**	**0.145**	**0.267**	**0.282**
Dambulla-Radish	**7**	**0.154**	**0.201**	**0.824**	**0.305**
Dambulla-Kohlrabi	**8**	**0.211**	**0.956**	**0.415**	**0.254**
Dambulla-Cauliflower	**9**	**0.142**	**0.745**	**0.728**	**0.154**
Dambulla-Collard greens	**10**	**0.123**	**0.924**	**0.491**	**0.245**
Welimada-Cabbage	**11**	**0.154**	**0.197**	**0.232**	**0.268**
Welimada-Radish	**12**	**0.186**	**0.181**	**0.752**	**0.242**
Welimada-Kohlrabi	**13**	**0.165**	**0.878**	**0.374**	**0.124**
Welimada-Cauliflower	**14**	**0.523**	**0.864**	**0.845**	**0.159**
Welimada-Collard greens	**15**	**0.101**	**0.979**	**0.542**	**0.216**

3.1 Comparison of Recorded heavy Metal Concentration of test Leafy vegetables with WHO Standard values

Table 2. Comparison of recorded heavy metal concentration of cruciferecae vegetables with WHO standards values

Vegetable	Concentration(ppm)			
	Lead	Copper	Zink	Chromium
WHO standard	**0.01**	**2.000**	**5.000**	**0.3**
Cabbage	**0.175**	**0.172667**	**0.241333**	**0.26**
Radish	**0.167667**	**0.184333**	**0.700**	**0.266**
Kohlrabi	**0.191667**	**0.872333**	**0.384667**	**0.241**
Cauliflower	**0.275667**	**0.840333**	**0.742333**	**0.2236667**
Collard greens	**0.125667**	**0.966**	**0.529333**	**0.2866667**

3.1.1 Comparison of Recorded Lead Concentrations (ppm)

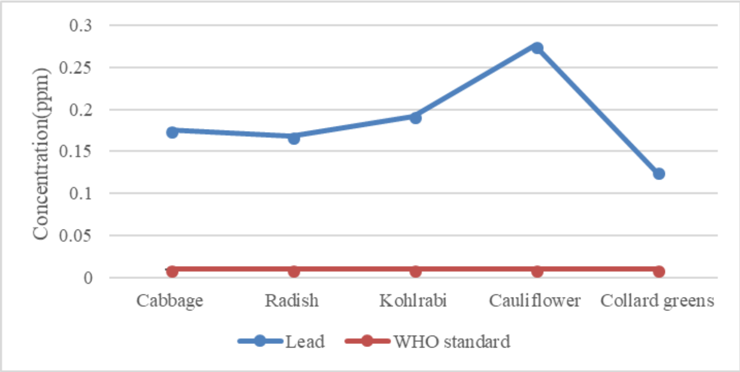

Fig 3. Comparison of Recorded Lead Concentrations (ppm)

Figure 3 shows that the estimated concentrations of Pb in test vegetables (cabbage, Radish, Kohlrabi, Cauliflower and collard Greens).According to this graph it can be suggested that the consumption of average amounts of these contaminated vegetables pose a health risk for the consumers as the values obtained are above the WHO limits for the PB intake (WHO has set health – based guideline values for Pb at 0.01 mg/l)

3.1.2 Comparison of Recorded Copper Concentrations (ppm)

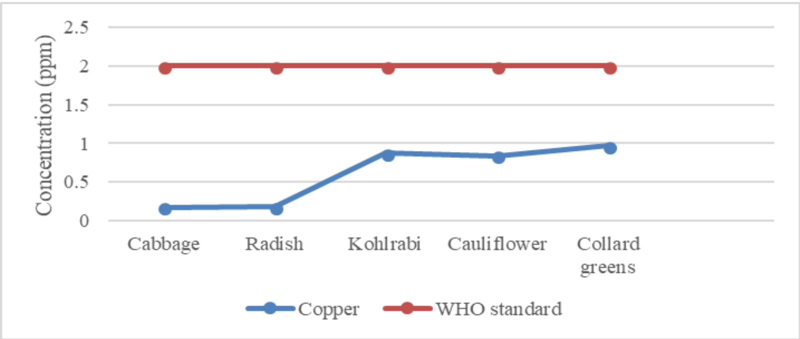

Fig 4. Comparison of Recorded Copper Concentrations (ppm)

Figure 4 represented that the estimated concentrations of Cu in test vegetables (cabbage, Radish, Kohlrabi, Cauliflower and collard Greens).According to the graph it is clear that the consumption of average amounts of the these contaminated vegetables does not pose a health risk for the consumers as the values obtained are below the WHO limits for the PB intake (WHO has set health – based guideline values for Cu at 2 mg/l)

3.1.3 Comparison of Recorded Chromium Concentrations (ppm)

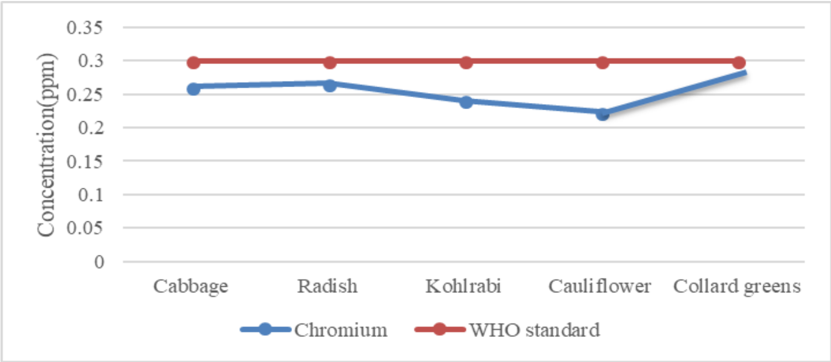

Fig 5. Comparison of Recorded Chromium Concentrations (ppm)

The estimated concentrations of Cr in test vegetables (cabbage, Radish, Kohlrabi, Cauliflower and collard Greens) shown in figure 5. It can be suggested that the consumption of average amounts of these contaminated vegetables does not pose a health risk for the consumers as the values obtained are below the WHO limits for the Cr intake (WHO has set health – based guideline values for Cr at 0.3 mg/l).

3.1.4 Comparison of Recorded Zinc Concentrations (ppm)

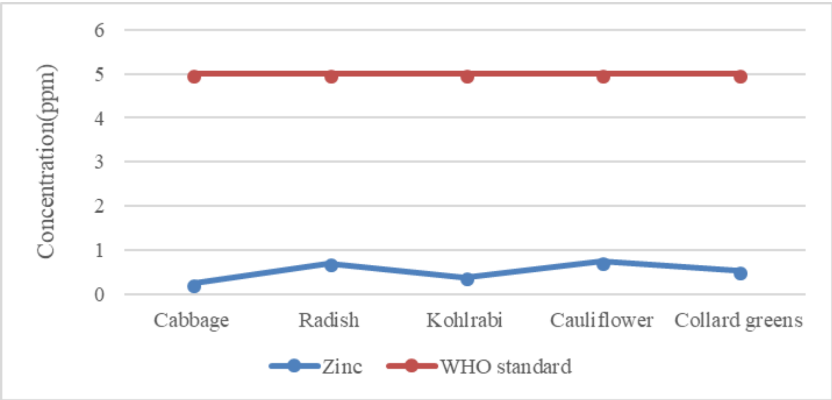

Fig 6. Comparison of Recorded Zinc Concentrations (ppm)

The estimated concentrations of Zn in test vegetables (cabbage, Radish, Kohlrabi, Cauliflower and collard Greens) illustrated in figure 6. the results revealed that the consumption of average amounts of the these contaminated vegetables does not pose a health risk for the consumers as the values obtained are below the WHO limits for the Zn intake (WHO has set health – based guideline values for Zn at 5 mg/l).

3.2 Statistical analysis

This test was performed to show that heavy metal concentration are significantly varied among the test vegetable and to find the highly contaminated area Vs vegetable combination (Appendix A) for each tested metal.

3.2.1 Variation of average rank of Lead

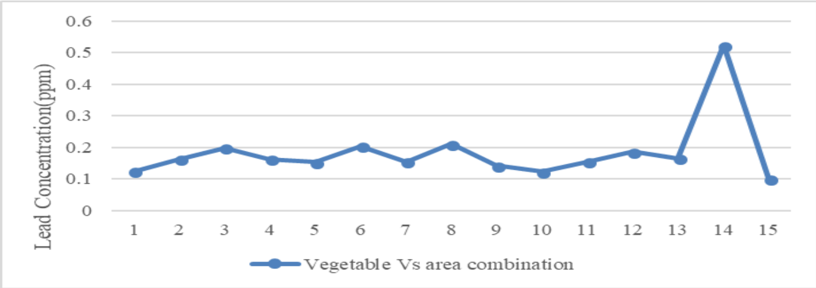

Fig 7. Variation of average rank of Lead

Figure 7 shows the variation of average ranks of Lead concentration with test vegetable Vs area combination. P- value is less than alpha value. Therefore, H0 is rejected and pair wise comparison is performed. The pair wise comparison is applied to the area Vs vegetable combinations. The14th pair is showing the highest lead concentration. Pairs 1, 5, 7, 9, 10, 11and 15 (Appendix A) are not showing any significant differences to the 14th pair.

3.2.2 Variation of average rank of Copper

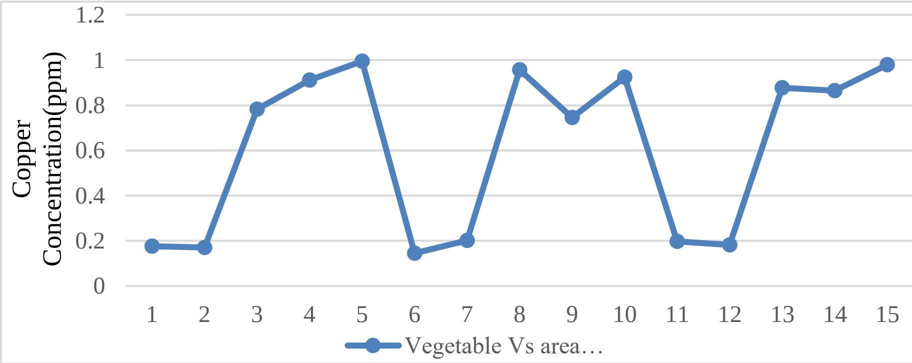

Fig 8. Variation of average rank of Copper

Figure 8 shows the variation of average ranks of Copper concentration with test vegetable Vs area combination P- value is less than alpha value. Therefore, H0 is rejected and pair wise comparison is performed. The pair wise comparison is applied to the area Vs vegetable combinations. The 5th pair is showing the highest copper concentration. Pairs 1, 2, 6, 7, 9, 11 and 12 (Appendix A) are not showing any significant differences to the 5th pair.

3.2.3 Variation of average rank of Chromium

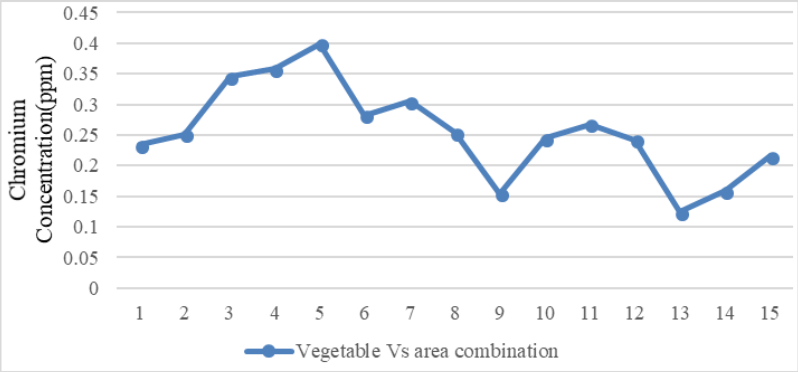

Fig 9. Variation of average rank of Chromium.

Figure 9shows the variation of average ranks of Chromium concentration with test vegetable Vs area combination P- value is less than alpha value. Therefore, H0is rejected and pair wise comparison is performed. The pair wise comparison is applied to the area Vs vegetable combinations. The 5th pair is showing the highest chromium concentration. Pairs 1, 9, 10, 12, 13, 14and 15 (Appendix A) are not showing any significant differences to the 5th pair.

3.2.4 Variation of average rank of Zinc

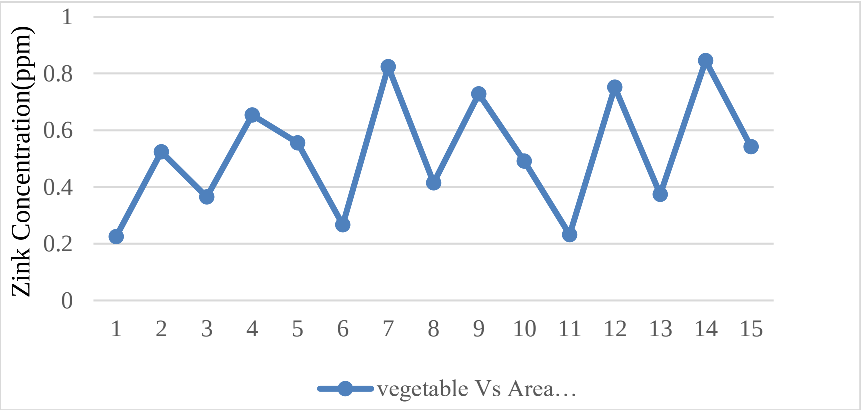

Fig 10. Variation of average rank of Zinc

Figure 10 shows the variation of average ranks of Zinc concentration with test vegetable Vs area combination P- value is less than alpha value. Therefore, H0is rejected and pair wise comparison is performed. The pair wise comparison is applied to the area Vs vegetable combinations. The 7th pair is showing the highest zinc concentration. Pairs 1, 3, 6, 8, 10, 11and 13 (Appendix A) are not showing any significant differences to the 7th pair.

Conclusion

The current study has created data on heavy metal contamination in test vegetable variant and variation of heavy metal contamination in selected study areas. The results of statistical analysis show that heavy metal contamination is diverse among the test vegetable and areas. From the assessed concentration of the lead of selected vegetable variants can be proposed that the consumption of average amounts of these contaminated vegetables poses a health risk for the buyers as the values obtained are bigger than WHO limit. For the Pb intake estimated concentration of Cu, Zn and Cr of selected vegetable variants can be proposed that the intake of average amount of these contaminated vegetables do not pose health risk for the consumers as these the values obtained are below to the WHO Limits. Finally it can be concluded that the intake amount of these contaminated vegetable poses a health risk for the consumers.

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Appendix A : Kruskal-Wallis Test

For all possible pairs, the hypotheses to be tested are;
H0: There is no significant difference between pairs
H1: There is significant difference between pairs
Decision Rule: if p value < α value, then reject H0

Kruskal-Wallis Test: Lead concentration versus Vegetable Vs area combination

Kruskal-Wallis Test on Lead concentration (ppm)

Vegetable Vs area

Combination	N	Median	Ave Rank	Z
1	1	0.1240	3.0	-1.16
2	1	0.1630	9.0	0.23
3	1	0.1990	12.0	0.93
4	1	0.1620	8.0	0.00
5	1	0.1530	5.0	-0.69
6	1	0.2040	13.0	1.16
7	1	0.1540	6.5	-0.35
8	1	0.2110	14.0	1.39
9	1	0.1420	4.0	-0.93
10	1	0.1230	2.0	-1.39
11	1	0.1540	6.5	-0.35
12	1	0.1860	11.0	0.69
13	1	0.1650	10.0	0.46
14	1	0.5230	15.0	1.62
15	1	0.1010	1.0	-1.62
Overall	15		8.0	

Kruskal-Wallis Test: Copper concentration versus Vegetable Vs area combination

Kruskal-Wallis Test on Copper concentration (ppm)

Vegetable Vs area

Combination	N	Median	Ave Rank	Z
1	1	0.1760	3.0	-1.16
2	1	0.1710	2.0	-1.39
3	1	0.7830	8.0	0.00
4	1	0.9120	11.0	0.69

5	1 0.9950	15.0	1.62
6	1 0.1450	1.0	-1.62
7	1 0.2010	6.0	-0.46
8	1 0.9560	13.0	1.16
9	1 0.7450	7.0	-0.23
10	1 0.9240	12.0	0.93
11	1 0.1970	5.0	-0.69
12	1 0.1810	4.0	-0.93
13	1 0.8780	10.0	0.46
14	1 0.8640	9.0	0.23
15	1 0.9790	14.0	1.39
Overall	15	8.0	

Kruskal-Wallis Test: Chromium concentration versus Vegetable Vs area combination

Kruskal-Wallis Test on Chromium concentration (ppm)

Vegetable Vs area Combination	N Median	Ave Rank	Z
1	1 0.2340	5.0	-0.69
2	1 0.2510	8.0	0.00
3	1 0.3450	13.0	1.16
4	1 0.3580	14.0	1.39
5	1 0.3990	15.0	1.62
6	1 0.2820	11.0	0.69
7	1 0.3050	12.0	0.93
8	1 0.2540	9.0	0.23
9	1 0.1540	2.0	-1.39
10	1 0.2450	7.0	-0.23
11	1 0.2680	10.0	0.46
12	1 0.2420	6.0	-0.46
13	1 0.1240	1.0	-1.62
14	1 0.1590	3.0	-1.16
15	1 0.2160	4.0	-0.93
Overall	15	8.0	

Kruskal-Wallis Test: Zinc concentration (ppm) versus Vegetable Vs area combination

Kruskal-Wallis Test on Zinc concentration (ppm)

Vegetable Vs Area Combination	N	Median	Ave Rank	Z
1	1	0.2250	1.0	-1.62
2	1	0.5240	8.0	0.00
3	1	0.3650	4.0	-0.93
4	1	0.6540	11.0	0.69
5	1	0.5550	10.0	0.46
6	1	0.2670	3.0	-1.16
7	1	0.8240	14.0	1.39
8	1	0.4150	6.0	-0.46
9	1	0.7280	12.0	0.93
10	1	0.4910	7.0	-0.23
11	1	0.2320	2.0	-1.39
12	1	0.7520	13.0	1.16
13	1	0.3740	5.0	-0.69
14	1	0.8450	15.0	1.62
15	1	0.5420	9.0	0.23
Overall	15		8.0	