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Assessment of Heavy Metals Content in Edible Vegetable of Kangkung (*Ipomaea*) Grown on Tondano Sediments-Based Fly Ash Bottom Ash (FABA) Mixtures

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ABSTRACT

The absence of heavy metals in edible vegetable is necessary for their safe consumption. The present study aims to assess the heavy metals content in the edible vegetable Kangkung grown on Tondano Sediments-based fly ash bottom ash (FABA) mixtures. The research was conducted by both purposive sampling method for the sediment, water hyacinth, and FABA samples, and complete randomized design for Kangkung planting in five treatments (A: sediment, B: FABA, C: sediment:FABA (25:75), D: sediment:FABA (50:50), and E: sediment:FABA (75:25)) in three replicates totaling 15 experimental pot samples. Water hyacinth was used as green fertilizer in treatments C – E. Sediment, FABA, and green fertilizer of water hyacinth were analyzed for plant nutrients (N, P, and K), pH, organic carbon, and texture. The effect of the treatments on the growth of Kangkung was assessed by observing the plant height, number of leaves, and plant fresh weight up to six weeks after planting. The heavy metals contents were determined by atomic absorption spectrophotometry (AAS). Results showed that the treatments did not significantly affect the growth of Kangkung except for treatment B (FABA) which significantly affected plant height at weeks 2 and 5. Heavy metal analysis detected arsenic, cadmium, and chromium in Kangkung after harvest, although in quantities below the Sediment Quality Guidelines (SQGs) limits. These findings suggest that sediments-based FABA mixtures may play a role in heavy metal absorption by Kangkung, the concentrations of these metals are not sufficient to affect the safe consumption of this vegetable.

Keywords: Heavy metals, Tondano Sediment, Fly ash, Bottom ash, Edible vegetable.

Introduction

The presence of heavy metals at varying concentrations in the aquatic ecosystems may be due to biogeochemical cycling and anthropogenic activities. The most potentially harmful of these heavy metals are lead, mercury and cadmium.[1] Heavy metal is generally used as a generic term for metals and metalloids associated with environmental pollution, toxicity and adverse effects on living organisms. They are considered the most important pollutants of the aquatic environment due to their toxicity and accumulation by aquatic organisms.[2] Aquatic contaminants such as heavy metals are not easily destroyed through the natural process of biodegradation, and therefore can accumulate in the environment.[3] Lake Tondano as a natural resource provides some functions to the local community. However, increasing population has placed a huge demand on the lake, which has resulted in some problems, such as sedimentation or shallowing of the lake, and environmental pollution.[4] The sedimentation caused by land erosion in the catchment area and lake drainage basin has resulted in the accumulation of water hyacinth (*Eichornia crassipes*) an aquatic weed, which has covered some parts of the lake.

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Excessive use of fertilizers on rice field, duck husbandry, household wastes (such as detergent, human feces), and fishery activities have contributed significantly to the high concentration of organic and inorganic wastes, including heavy metals in Lake Tondano.[5] Heavy metals in water bodies are a threat to living organisms who depend on them for source of water. Heavy metals such as mercury (Hg), lead (Pb), cadmium (Cd), copper (Cu), etc, accumulate in the environment (water, biota, sediments) where they find their way into living organisms, causing toxic effect to the organisms.[6] Content of Pb in the water of Lake Tondano, especially in the District of Remboken (at the villages of Kaima, Paslaten and Leleko), has exceeded the limit set in the water quality guidelines.[7] Research have shown that water hyacinth could decrease the content of heavy metals like Cu, Cd, and Pb within a few days.[8,9] Heavy metals such as, nickel, copper, chromium, lead, and zinc were observed to accumulate in varying and sometimes very low but measurable concentrations in water and sediment samples across creeks, and these heavy metals have been demonstrated to alter the presence of important phytochemicals, and affect the antioxidants activity of vegetables grown in the area.[10,11] Therefore, caution should be exercised in the therapeutic use of plants grown in such areas due to the potentially high concentrations of heavy metals in such plants.[12]
The amount of heavy metals absorbed from the soil most times depends on the geochemical characteristics of the soil, the bioavailability of the element, and the ability of the plant to accumulate the metals. Aside from soil, plants also acquire these elements from atmospheric dust, fertilizers, herbicides, and rainwater.[13] With increasing human activities that generate a large volume of both industrial and domestic wastes daily coupled with poor wastes management, and deteriorating climatic conditions, plants tend to accumulate more of these heavy metals, and this portend danger to both soil flora and fauna, including man who represents the final consumer at the top of the food chain.[14] Heavy metal toxicity represents one of the most environmentally induced health challenges and a major pollutant worldwide.[15]

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Sediment potentially serves as an important source of chemical constituents of the aquatic system. As a result, sediment may play a key role in investigating water-pollution sources and effects. Investigating chemical contaminants through sediment collection is a process, which needs to be deeply understood as there, is limited understanding of sediments at the moment. The situation is complicated by the limited sampling techniques and the absence of guidelines for sediment quality in Indonesia presently. To overcome these limitations, it is important to obtain complete sampling tools, and use the sediment guidelines from other countries like the United States of America and Canada.[16]

Researchers have proposed that Lake Tondano should be paid attention by the Government and stakeholders. Studies that have been done on Lake Tondano include the investigation of the growth of Pakcoy, seasonal plants, and nuts using organic fertilizer from water hyacinth and sediment of Lake Tondano.[4,17-19] Study on Fly ash of charcoal obtained from electric power plant in Lake Tondano showed that the fly ash consisted of silicon oxide (SiO_2), aluminium oxide (Al_2O_3), and iron oxide (Fe_2O_3) as the main component. About 55-85% of the ash produced by the charcoal was fly ash, and the remainder was bottom ash.[20] Although, research on the effect of Lake Tondano sediment based on Fly Ash Bottom Ash (FABA) using water hyacinth as green fertilizer on the growth of Kangkung (*Ipomoea aquatica* F.) has been conducted, it was therefore necessary to investigate the sediment of Lake Tondano as plant media, water hyacinth as green fertilizer by using other waste namely Fly Ash Bottom Ash (FABA) from electric steam power plant of Sulut 3, in an effort to produce a technologically useful product. These materials, which were previously wastes materials in the environment, are now being turned into useful products. Therefore, this study aimed to assess the heavy metals content of edible vegetable of Kangkung (*Ipomaea*) grown on Tondano Sediments based fly ash bottom ash (FABA) mixtures in attempts to determine their safety level.

Materials and Methods

Materials

Materials used in the research include sediment, water hyacinth, fly ash bottom ash (FABA), sand, and Kangkung seed.

Study design

Purposive sampling method was used for sediment, water hyacinth, and FABA collection. Complete Randomized Design (CRD) in five treatments and three repetition was used to get 15 experimental pots for Kangkung on the field. The procedures consisted of:

a. Pre-survey

Pre-survey was conducted at the locations where samples of Lake Tondano sediment, water hyacinth, and FABA at PLTU Sulut 3 of Kema were collected.

b. Sampling

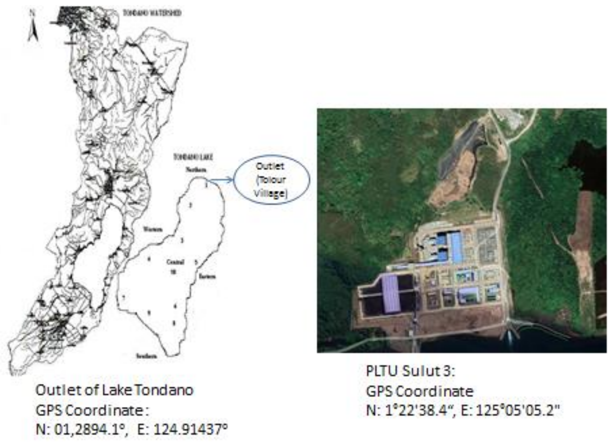
Sediment and water hyacinth samples were collected at the outlet of Lake Tondano (i.e. Toulour Village of Minahasa Regency) with the following GPS coordinate; N: 01'2894.1º, E:124.91437º. FABA samples were collected from electric steam power plant of Sulut 3 (at Kema Village of North Minahasa Regency) with GPS coordinate N: 1°22'38.4", E: 125°05'05.2" (Figure 1). The sediment was taken from the outlet of Tondano Lake by boat and dredged by Grab sampler to the base of the lake. The samples were placed in suitable containers until ready for analysis.

Preparation and analysis of sediment

The sediment was spread on a tarpaulin and sun-dried for 14 days, and thereafter analyzed for plant nutrients (N, P, and K), pH, organic carbon, and texture as highlighted below:

Determination of plant nutrients: N, P, and K contents were determined using Bray 1 Method. *Determination of pH:* pH was determined with a pH meter. *Determination of organic carbon:* Two 25 g portions of the sediment were measured and placed into separate porcelain dishes of known weight (previously dried at 105ºC). The samples were heated in the oven at 375ºC for 16 hours, after which they were cooled and their weights determined. The organic carbon was then calculated using the IPB Formula as previously described.[21]

Determination of grain size: Grain size of the sediment was determined using the pipette method as follows; 10 g of soft air-dried soil (2 mm sieved) was weighed, and 30-40 mL of 6% H_2O_2 solution was added. The mixture was left overnight, after which 10 mL of 30% H_2O_2 solution was added. The mixture was then heated in the oven at 105ºC until the H_2O_2 had evaporated completely. To the dry soil was added 45 mL of 0.4 N HCl, left overnight, and then washed three times with HCl solution in order to remove its gaseous contents, and water. The mixture was filtered, and the filtrate was treated with $AgNO_3$, 20 mL of peptizing agent 0.1 N $Na_4P_2O_7.10H_2O$ was added, and the mixture was heated directly with water for 30 minutes. It was then filtered through a 50 µm sieve. The sand (diameter >0.05mm) was poured into a beaker and dried at 105ºC until a constant weight was reached. Grains between 0 and 50 µm in size were pipetted, the mixture was shaken for one minute, and the grains were again pipetted directly at a depth of 15cm (as 0 minute). The mixture was then weighed, dried in the oven for 3 hours 31 minutes, after which time it was pipetted at a depth of 5 cm. The weight of each grain was then determined and converted into a percentage, after which the textural classes were determined using the USDA-Texture Triangle.[21] Particles were grouped by size as follows: gravel (2-50 mm), sand (2-0.05 mm), silt (0.05-0.002 mm), and clay (< 0.002 mm).[21]



(A) **(B)**

Figure 1: Sampling locations **(A)** sediment at outlet of Lake Tondano, and **(B)** FABA at PLTU Sulut 3

Preparation and analysis of water hyacinth

The water hyacinth has cut into small sizes, air-dried for 7 days, and incubated for 7 days when was decayed and became brown coloured. The composition of green fertilizer of the water hyacinth was determined.

Preparation and analysis of FABA (Fly Ash Bottom Ash)

FABA was taken from the landfill of Electric Steam Power Plant of Sulut 3, filled into sacks, and brought to the laboratory. They were dried, and then mixed with certain amount of sediments and sand. The composition of both fly ash and bottom ash was determined.

Planting of Kangkung

The mixture of sediment, water hyacinth and FABA was prepared as follows; A = sediment, B = fly ash bottom ash (FABA), C = mixture of sediment and FABA (25:75) with water hyacinth green fertilizer at 30 t/Ha dozen, D = mixture of sediment and FABA (50:50) with water hyacinth green fertilizer at 30 t/Ha dozen, E = mixture of sediment and FABA (75:25) with water hyacinth green fertilizer at 30 t/Ha dozen. Every pot had 3 kg weight of C, D, and E at ratio 1:1:1. Treatment A (sediment) and B (FABA) served as the controls. The mixtures of C, D, and E had water hyacinth green fertilizer at 30 t/Ha equivalent to 45 g/pot. The pots were watered, incubated for two weeks, then some seeds

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of Kangkung were planted in the pot. After seven days, the plants were thinned to allow 2-3 plants per pot, the plants were maintenance and observed for the following growth parameters: height of plant and number of leaves per week (for six weeks). The plants were harvested at maturity, weighed, then the composition of the plant media were analysed after harvesting.

Heavy metals analysis of Kangkung plant
Each plant harvested from the pot was washed, all plant parts were weighed, the plant was cut into small size, and placed into a plastic sack according to each treatment. The plants were observed for descriptive characteristics, then analyzed for heavy metals content.[21] The heavy metals analyzed include Arsenic (As), Cadmium (Cd), and Chromium (Cr) using atomic absorption spectrophotometry (AAS) according to standard procedures.

Statistical analysis
Data were analyzed using one-way Analyses of Variance (ANOVA), followed by Least Significant Difference (LSD) Test at 95% confidence level. Probability value (P-value) less than 0.05 was considered significant.

Results and Discussion

Composition of each sample
The composition of the samples was analyzed in two phases: (I) Before planting which consisted of fly ash (FA), bottom ash (BA), green fertilizer of water hyacinth, and lake sediment, and (II) After planting which consisted of sediment (sample A), FABA (sample B), mixtures of sediment and FABA (samples C, D, and E). Variables analyzed include pH, organic carbon, and nutrients (N, P, and K). These variables were analyzed in order to determine the effect of anthropological activities on these samples. The results of these analyses are presented in Table 1.

Table 1: Characteristics of each sample before and after planting

Name of Sample	Kind of Variables				
	pH (H_2O)	**C-organic (%)**	**N soil** (%)	**P_2O_5** (ppm)	**K_2O** (ppm)
Before planting:					
-FA (*fly ash*)	7.51 (neutral)	1.81 (low)	0.16 (low)	2.80 (very low)	11.86
-BA (*bottom ash*)	7.13 (neutral)	1.39 (low)	0.12 (low)	3.15 (very low)	13.11
-PHEG (organic fertilizer of water hyacinth)	6.30 (neutral)	19.09 (very high)	1.44 (medium)	24.23 (very high)	26.00
-Lake Sediment	6.56 (neutral)	8.20 (high)	0.50 (medium)	18.65 (high)	15.53
After planting:					
- A (Sediment sample)	7.05	1.25 (low)	0.11 (low)	12.10 (low)	18.11
- B (FABA sample)	6.79	1.18 (low)	0.10 (low)	13.44 (low)	17.12
- C (Mixture sample 25:75)	6.70	1.04 (low)	0.09 (very low)	14.11 (low)	15.23
- D (Mixture sample 50:50)	6.78	1.04 (low)	0.09 (very low)	13.98 (low)	17.56
- E (Mixture sample 75:25)	7.01	1.11 (low)	0.10 (low)	12.87 (low)	18.98

Note: These values were based on the Criteria of Soil Research Centre of 1983 in Sinolungan[21]

The degree of soil acidity (pH), is one of the factors that affect plant's growth. pH value indicates the hydrogen ion (H^+) concentration in soils. Lower pH value indicates higher degree of acidity. Value of soil pH must be stabled, or around neutrality (6 – 7). Measurement of soil pH is aimed at providing some information about the soil condition whether acidic or alkaline.[22] Moderately acidic pH is necessary for absorption of metals from the soil to the plants. Acidic soil would dissolve minerals and metals in the soil including toxic metals.[23] The result of pH determination as presented in Table 1 showed that all samples both before and after planting had pH values around 6-7, indicating that the samples are in neutral condition.
Organic carbon (C-organic) of the soil is one of the indicators of soil fertility quality. The organic carbon content is an important standard in soil cultivation. Excessively high organic carbon content could affect the development of soil microorganisms due to increased C-organic compared to the content of total soil Nitrogen (N_{total}). This condition reduces protein production and inhibit the activities of microorganisms.[24] It was therefore necessary to assess the organic carbon content in order to predict N-total, a variable that help assess the level of decay and decomposition of organic matter, and the existing soil nutrients.

The results of organic carbon analysis as presented in Table 1 showed that all samples after planting were categorized as low in organic carbon, whereas the samples before planting were categorized as low to very high in organic fertilizer of water hyacinth, this is probably due to fact that the organic matter in these samples is not used up yet. The fertilizer serves as nutrient source needed for plant growth and development. N, P, and K content in FABA before planting were categorized as very low to low, in organic fertilizer of water hyacinth, these nutrients were medium to very high, while in lake sediment, they were medium to high. In this study, all of the samples were mixed and used as media for Kangkung planting, so that the plant can draw nutrients from the mixture of these samples.

Kangkung growth for six weeks after planting
The outcomes of Kangkung growth in the five treatments (A, B, C, D, and E) in the pot experiments conducted by complete randomized design on the field are presented below:

1. *Plant height*

The average height of Kangkung is presented in Table 2. Analysis showed that there was significance effect of the treatment on plant

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height at weeks 2 and 5 except for treatments B and C which showed no effect at week 2. The lowest plant height was recorded in the treatment with FABA only. The relationship between groups and within groups could be seen from the result obtained at week 5, FABA treatment had the lowest value, and had no significant effect on plant height, whereas treatments A, C and E significantly affected plant height. This indicate that FABA alone is not sufficient to support plant growth, as it does not possess the needed nutrients and physicochemical characteistics necessary for adequate growth and development of the plant.

2. Number of leaves

Table 3 shows the average number of leaves of Kangkung plant grown on the different samples. Analysis showed that there was no significant effect of the treatment on the number of leaves, however Kangkung plant on treatment B (FABA) had the lowest number of leaves, while treatment A (Lake sediment) had Kangkung plant with the highest number of leaves. This indicates that the plant would grow well on soil that facilitates nutrients absorption.

Table 2: Average height of Kangkung plant (cm)

Treatment	1WAP	2 WAP	3 WAP	4 WAP	5 WAP	6 WAP
A (Sediment)	3.53	7.44b	10.73	12.8	23.48b	16.45
B (FABA)	1.66	3.49a	5.8	6.37	7.7a	8.26
C (Sediment:FABA 25:75)	2.41	4.03a	6.44	9.61	31.18b	14.74
D (Sediment:FABA 50:50)	4.22	7.52b	7.54	28.78	13.1ab	20.54
E (Sediment:FABA 75:25)	3.18	6.96b	9.21	14.74	23.52b	25.46
LSD 5%	-	2.49	-	-	10.4	-

WAP = Week after planting, LSD = Least Significant Difference (LSD) Test

Table 3: Average number of leaves of Kangkung plant grown on the different soil media

Treatment	1WAP	2WAP	3WAP	4WAP	5WAP	6WAP
A (Sediment)	4	7	6	7	11	12
B (FABA)	3	5	5	5	6	6
C (Sediment:FABA 25:75)	2	3	5	6	7	8
D (Sediment:FABA 50:50)	3	6	6	7	10	10
E (Sediment:FABA 75:25)	2	3	6	6	11	13

WAP = week after planting, LSD = Least Significant Difference (LSD) Test

3. Fresh weight of plant

The average weight of the plant grown on the different treatment are presented in Table 4. The result showed that the treatment have no significant effect on the fresh weight of the plant. The highest plant weight was observed in treatment A (sediment). This indicate that the sediment has the physicochemical characteristics that support the growth and development of the plant.

Heavy metals content of the plant after harvest

The contents of heavy metals (As, Cd, and Cr) of Kangkung after being harvested from the various treatments are highlighted below:

1) *Arsenic (As) content in Kangkung vs SQGs*

The content of As in Kangkung was in the range of 0.04 – 0.43 mg/kg (Figure 2), where the highest content was found in plants grown on treatment B, i.e., only FABA sample. This implies that FABA was derived from coal combustion products which originally have high amount of arsenic, although the value was below the limit set for SQGs. Fly ash contained oxides of some elements, where the main elements are Al, Si, Ca, Fe, Mg, Na, K, S, P, Ba, Sr, in addition to small amounts of Arsenic (As), Boron (B), Cadmium (Cd), Cobalt (Co), Chromium (Cr), Cesium (Cs), Copper (Cu), Galium (Ga), Mercury (Hg), Lantanum (La), Manganese (Mn), Molybdenum (Mo), Nickel (Ni), and lead (Pb). This ash contained almost all the essential elements which are needed by the plant, except N and C.[25]

2) *Cadmium (Cd) content in Kangkung vs SQGs*

The content of Cd in Kangkung was in the range of 0.005 – 0.02 mg/kg (Figure 3), with the highest content found in the plant grown on treatment A, i.e., only Lake Tondano sediment sample. The high cadmium content in this sample could be a result of increased human activities around the lake. Despite the high Cd value, it was still below the SQGs limit. According to BSN (2009),[26] the safe tolerance limit for Cd in food and vegetables is 0.2 mg/kg.

3) *Chromium (Cr) content in Kangkung vs SQGs*

The content of Cr in Kangkung was 0.07 mg/kg (Figure 4) for all the treatments and this was below the SQGs limit. Heavy metals accumulation in plant depends on the content of metals in the soil, the kind of metals, soil chemicals, soil pH, and plant species. The soil pH has the highest effect on the solubility of metals in soils.[27] The content of heavy metals in Kangkung at harvest did not exceed the SQGs limit, which means that the plants grown on these samples are still safe for human consumption. Heavy metals contents in plants (although in small amount) are derived from sources like pesticides, and inorganic fertilizers used on agriculture land, household materials, and others. Kangkung is one of many plants that could easily absorb heavy metals from the soil.[28] Therefore, the use of Lake Tondano sediment-based FABA and green fertilizer of water hyacinth in an experimental pot model had effect on Kangkung growth. Statistical analysis showed that the effect of these treatments was significant for the height of Kangkung plant at the second and fifth week after planting, whereas the number of leaves and fresh weight of the plant were not significantly affected. The findings from this study have shown that use of FABA without being mixed with sediment or green fertilizer is not a good media for healthy growth and development of plants. However, the use of water hyacinth as the green fertilizer has a huge impact on the content of heavy metals in edible vegetable, such as Kangkung.

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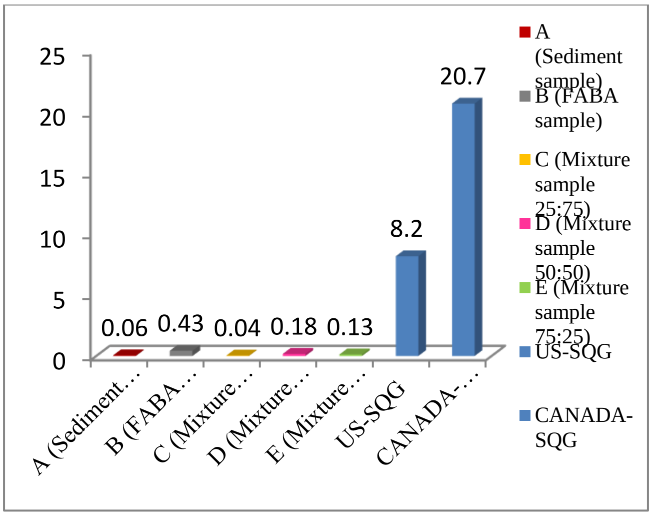

Figure 2: Arsenic (As) content of Kangkung vs SQGs

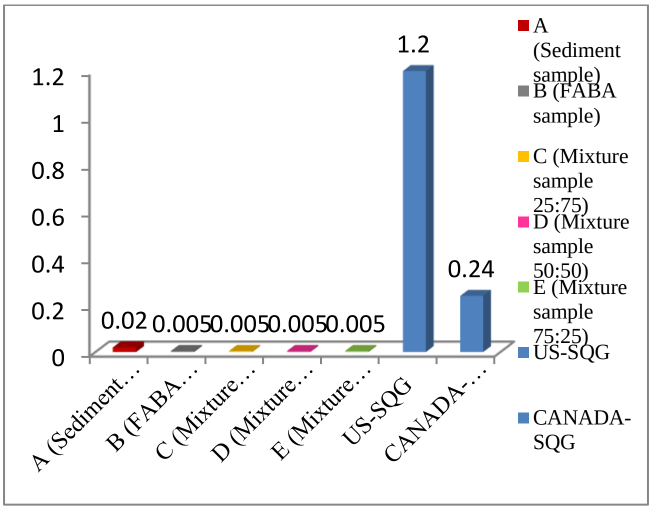

Figure 3: Cadmium (Cd) content of Kangkung vs SQGs

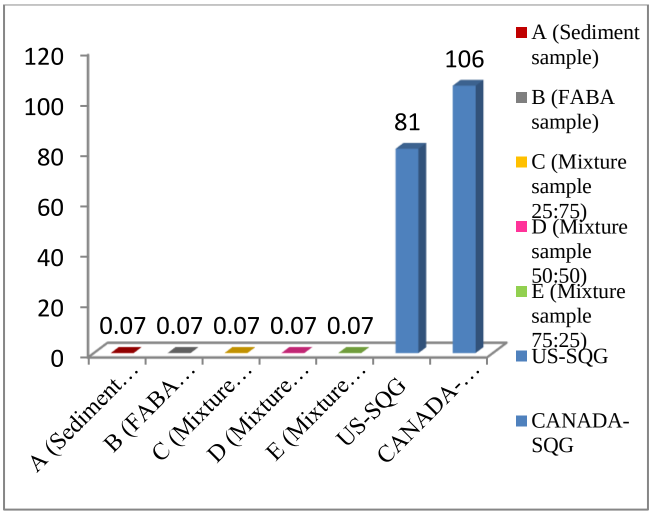

Figure 4: Chromium (Cr) content of Kangkung vs SQGs

Table 4: Average fresh weight of Kangkung grown on the different soil media

Treatment	Fresh Weight of Plant (g)
A (Sediment)	25
B (FABA)	17
C (Sediment:FABA 25:75)	11
D (Sediment:FABA 50:50)	20
E (Sediment:FABA 75:25)	22

Conclusion

The present study has therefore demonstrated the presence of heavy metals (As, Cd, and Cr) in edible vegetable Kangkung (*Ipomaea*) grown in Tondano Sediments based fly ash bottom ash (FABA) mixtures, albeit in small quantities that is safe for human consumption. However, there should be caution in the use of these plant media, i.e. sediment-based FABA in high concentration.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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