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Heavy Metal Levels and Target Hazard Quotients of *Ipomoea aquatica* Grown in Soils Applied with Stabilized Biosolids

Eva R. Orlina[1,*], Ariel G. Mactal[2], Purisima P. Juico[2], Maria Luisa T. Mason[2], Danila S. Paragas[3], Jacqueline D. Maquirang[4]

[1]Department of Plant Science, College of Agriculture, Forestry and Environmental Sciences, Aklan State University, Philippines
[2]Department of Soil Sciences, College of Agriculture, Central Luzon State University, Philippines
[3]Department of Chemistry, College of Arts and Sciences, Central Luzon State University, Philippines
[4]Production Engineer, Boracay Island Water Company Incorporated, Philippines

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Abstract Though rich in nutrient and organic materials, biosolids contain heavy metals, organic pollutants, and substances of emerging concerns. This study evaluated the effects of stabilized biosolids from Sewage Treatment Plant in Boracay Island on selected soil properties, heavy metals and target hazard quotients associated with the *Ipomoea aquatica* consumption. The field experiment was laid out in a randomized complete block design (RCBD) in three replications. A total of 21 experimental microplots with an area of 1 m × 2 m were used. Treatments were: Natural Field Condition (T_1), Natural Stabilization (T_2), Photocatalytic Stabilization (T_3), Effective Microorganism Stabilization (T_4), Indigenous Microorganism Stabilization (T_5), Vermistabilization (*Eudrilus eugeniae* + *Gliricidia sepium* leaves + *Oryza sativa* straw) (T_6) and Inorganic Fertilizer Recommended Rate (30-30-30 kg NPK/ha) (T_7). Results showed that the levels of heavy metals cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn) in the stabilized biosolids and soils were within the permissible limits set by the Department of Environment and Natural Resources Administrative Order (DAO) 2013-22. The biosolids application did not show phyto availability of heavy metals. The target hazard quotient (THQ) values of heavy metals in the plants were <1, indicating no harmful effects on the consumers' lifetime.

Keywords Heavy Metals, Target Hazard Quotient, Stabilized Biosolids, *Ipomoea aquatica*

1. Introduction

The possible utilization of the huge volume of biosolids from the wastewater treatment plant (WWTP) in Boracay Island, a world-renowned tourist destination in Aklan, Philippines poses a significant challenge to the Province agricultural ecosystem. Biosolids are nutrient-rich organic materials resulting from domestic sewage treatment in a treatment facility. When treated and processed, these residuals can be recycled and applied as fertilizer to improve and maintain productive soils and stimulate plant growth [1-2]. Analysis of biosolids from the source contains 28.82 % organic matter, 2.26 % nitrogen, 0.002 % phosphorus, and 0.16 % potassium [3]. As reported by EPA (2000), the nutrients in biosolids offer several advantages over inorganic fertilizers since they are organic and are released slowly for growing plants. These organic nutrients are less water-soluble due to the presence of fats and waxes and, therefore, are less likely to leach into groundwater or runoff into surface water [1,4]. However, notwithstanding the indisputable advantages of applying biosolids in agriculture, biosolids involve some serious threats. Among these are the presence of pathogens like protozoa, parasitic ova, *Salmonella spp*., *Shigella spp*., and *Escherichia coli*, organic pollutants, and heavy metals [5].

Stabilization of biosolids helps minimize the potential for odor generation, destroys pathogens (disease-causing organisms), and reduces the material's vector attraction potential [1]. One effective method of stabilizing the biosafety of biosolids is through the vermicomposting process. Earthworms feed readily upon the biosolids components, rapidly converting them into vermicompost [6], reducing the pathogens to safe levels, and ingesting and complexating the heavy metals. Other methods identified by the USEPA, according to Sullivan, Coger, and Barry [7], include adjustment of pH or alkaline stabilization, digestion, and heat drying. Kumar and Gopal [8] have recommended methods using effective microorganisms (EM) and indigenous microorganisms (IMO). In addition, stabilization through photocatalysis as an emerging technology in the decomposition of organic substances and pollutants has been recognized [9]. As reported by Orlina, et al. [3], the biosolids from the island contained heavy metals such as Pb – 3.24, Cd – 0.2, Ni – 0.26, Cu – 0.76, and Zn – 3.11 (µg/g). All the values were very low compared to the limits set by the Bureau of Agriculture and Fisheries Standards (BAFS), which are 5 µg/g for Pb, Cd, Ni, and Cu, and 333 µg/g for Zn respectively. Despite being classified as clean biosolids, proper handling, monitoring, and management should be practiced in dealing with pathogenic organisms and heavy metals from this source, including pathways of their movements and utilization [3]. Since heavy metals exist in their elemental forms, they cannot be degraded. They can bioaccumulate in the ecosystem, which poses a severe risk to human health due to their mutagenic, teratogenic, and carcinogenic effects [10].

An actual study in the agricultural ecosystem is necessary to assess the movement of heavy metals from the stabilized biosolids. Since different crops show different affinities to heavy metals. The water spinach (*Ipomoea aquatica* Forrsk.) is a common vegetable and a part of the staple diet in the Philippines [11]. *Ipomoea aquatica* showed significantly high accumulations of heavy metals like Sr, Cr, Sn, in plant tissues, particularly in the leaves and roots [12]. It has a well-known tendency to absorb lead, including radioactive isotopes [11]. Recycling the island's biosolids maximizes its potential as an indispensable resource in agricultural production, considering that the soil in the province, is old with a low nutrient level. Therefore, it is essential to identify the best management option to utilize the resource for such soil, improve agricultural productivity, and, at the same time, protect the consumer as well as the environment. Hence the study was conducted to determine the chemical properties and heavy metal content of biosolids, the inherent heavy metal in soil applied with stabilized material and evaluate the heavy metal target hazard quotients associated with the consumption of *Ipomoea aquatica* grown in biosolids-laden soil.

2. Materials and Methods

2.1. Study Site

The study area is located in Barangay Mangan, Banga, Aklan, 11.631781 °N latitude and 122.354795 °E longitude with a four-meter elevation above mean sea level. It is located 14 km northeast of Kalibo, the provincial capital of Aklan. The site has a total land area of 0.3 hectares. The soil at the site is Alimodian clay loam (*fine isohypherthermic*, *calcic halfultalf*) [13]. The climate is classified as Type III (no very pronounced maximum rain period, with a short dry season lasting only from one to three months, either from December to February or from March to May). The annual mean temperature is 27 ^{0}C, while the mean monthly rainfall ranges from 141 to 414 mm [14]. The area has been planted with rice and was previously used as pastureland for cattle before setting the experiment.

2.2. Soil Characteristic Determination

Before the study, a composite soil sample (0-30 cm) was collected from 10 different spots in the experimental site and was processed to determine physicochemical characteristics. After air-drying and pulverizing, the sample was passed through a 2-mm sieve. The following soil properties were determined: soil organic carbon, soil pH, total N, P, and K contents, according to the methods devised by Lui [15] and Spark as cited by Pansu [16]. Aside from collecting soil samples, a survey of the soil profile in the area was conducted to provide additional essential data and information for the study.

2.3. Experimental Design and Treatment

The experiment was laid out in a randomized complete block design (RCBD) in three replications. A total of 21 experimental microplots with an area of 1 m × 2 m were used. The treatments were based on the amount of nutrients in the soil and biosolids. The rate of 15 Mg ha^{1} stabilized biosolids on the dry weight basis was applied for Treatment 2 to Treatment 6. Treatments assignments were as follows: Treatment 1- Natural field condition (Control); Treatment 2- Natural stabilization; Treatment 3- Photocatalytic stabilization; Treatment 4- Effective microorganism stabilization; Treatment 5- Indigenous microorganism stabilization; Treatment 6-

Vermistabilization (*Eudrilus eugeniae* + *G. sepium* leaves + *O. sativa* straw); Treatment 7- Inorganic Fertilizer Recommended Rate (30-30-30 kg NPK/ha).

Treatments were designed to evaluate the following: a) Treatment 1- while g), no biosolids added, b) Treatment 2 (natural stabilization) was devised to determine the fate of biosolids upon 'natural aging' with time and by the natural microbial activity occurring in them; c) Treatment 3 (photocatalytic stabilization) was designed to evaluate the properties of biosolids after exposure to a photocatalyst TiO_2; d) Treatment 4 (effective microorganism stabilization) was used to determine the effect of using effective microorganism (bioaugmentation) in the stabilization process; e) Treatment 5 (indigenous microorganisms stabilization) was used to determine the effect of using indigenous microorganism (bioaugmentation) in the stabilization process; f) Treatment 6 (vermistabilization) was used to determine the applicability of the recommended biodegradation process and nutrient cycling methods in biosolids stabilization using other substrates *Gliricidia sepium* leaves and *Oryza sativa* straw and *Eudrilus eugineae* earthworm while g) Treatment 7 (inorganic fertilizer recommended rate) determined the performance of *Ipomoea aquatica* using commonly utilized synthetic fertilizers.

2.4. Land Preparation

The area was prepared two to three weeks before planted. The stabilized biosolids were applied in the area at a 1.5 kg/m^2 rate by incorporating the materials in the soil before seed sowing. Twenty-one (21) one meter wide by 2 m long plots, 20 to 30 cm high beds spaced 0.5 m apart were established. Irrigation and drainage canals were prepared to prevent waterlogging during excessive rainfall occurrence.

2.5. Crop Establishment and Harvesting of *Ipomoea aquatica*

Direct seeding of *Ipomoea aquatica seeds* (Chinese Upland *Ling-ling* Batch No. R15616 Lot No. CKU RS19865 with 99% purity) was done by sowing three seeds per hill in a triangular arrangement with a distance of 30 cm between rows and 25 cm between hills with three rows per plot. Harvesting was done on the 45^{th} day after sowing early in the morning or late in the afternoon. The plants were cut at the base with a sharp knife two to three cm above the ground level.

2.6. Water, Pests and Diseases Management

Water was applied immediately after planting until the soil profile was thoroughly wet. Thereafter, watering was done every day or every 2-3 days for 4-6 hours or until the soil was thoroughly wet. The crop was monitored regularly for pest infestations by looking into the growing points and on the underside of the leaves. Where possible, eggs and young larvae were quashed, and leaf miner-infested leaves were pruned. Moving from a mite-infested crop into an uninfested crop was avoided. The crop was monitored regularly for early disease symptoms. Infected plants showing systemic signs were rogued, and infected parts for localized diseases were carefully pruned away. When pruning was needed to be done, pruning tools were disinfected after being applied to use on every plant. Bacterial wilt and blight can be transmitted via pruning tools. A container for pruned plant materials was provided during pruning. The pruned diseased or infested plant parts were placed immediately inside the bag and were disposed properly to minimize inoculum dispersal to healthy plants.

2.7. Soil and Plant Analyses

At maturity, the shoots of the sample plants were harvested, weighed, washed thoroughly with tap water and then with deionized water, oven-dried at 70 ◦C for 48 h, ground, sieved, weighed, and prepared for analysis of nutrients and heavy metals. Similarly, soil from each microplot was sampled, air-dried, and ground to pass through a 2-mm sieve for chemical nutrients and heavy metal analysis [17]. The composite sample was brought to the Bureau of Soils and Water Management (BSWM) Department of Agriculture Regional Field Office 6 in Iloilo City for routine soil nutrient analysis. Total Kjeldahl nitrogen was determined as recommended by van Reeuwijk [18], available phosphorus (mg kg^{-1} soil) by Olsen and Sommer [19], while available K was determined using atomic emission spectroscopy [15].

The pH and soluble organic matter were obtained following the methods Liu and Lin [20] described. In brief, the pH was determined using a combined electrode in a 1:5 (w/v) solid-water suspension. Organic-matter content was analyzed by the potassium dichromate oxidation-titration method. Soil samples were digested using aqua regia or extracted using diethylenetriaminepentaacetic acid (DTPA) solution 0.005 M DTPA, 0.1 M triethanolamine (TEA), and 0.01 M calcium chloride (CaCl2), pH 7.3 [17].

Biosolids, soils, and plant samples for heavy metals Cu, Ni, and Zn analyses were brought to the Analytical Services Laboratory of Central Luzon State University in Science City of Muñoz, Nueva Ecija. The heavy metals were determined using Microwave Plasma Atomic Emission Spectrometry (MP-AES) Agilent Technology (2011). Biosolids, soils, and plant samples were submitted to the Regional Soils Laboratory in San Fernando, Pampanga, for Pb and Cd analyses. The samples were analyzed using Flame Atomic Absorption Spectrophotometry (Flame AAS). Soil extract preparation for total heavy metal (Cd, Cu, Ni, Pb, and Zn) concentration was prepared by digestion of the samples with aqua regia [HNO3.HCl (v/v = 1:3)] [21].

2.8. Growth in Height and Tiller Number of *Ipomoea aquatica*

Growth in height and tiller number of *Ipomoea aquatica* were gathered at 15 days after sowing (DAS), and the plot yield was obtained during harvest at 45 DAS. The occurrence of pests and diseases was accounted for during the study.

2.9. Estimated Daily Intake

The estimated daily intake (EDI) was used to calculate how much *Ipomoea aquatica* was taken by an adult for one day. The conversion factor, 0.10, was utilized to convert the dry weight (dw) basis of the samples into wet weight (ww), as suggested by Yacoob et al. [22]. The mean concentrations of the heavy metals in the treatments were used for the calculation of the estimated daily intake. The EDI (μg/kg/day) of *Ipomoea aquatica* that contains heavy metals Cd, Cu, Ni, Pb and Zn was measured using the following equation [23]

$$EDI = MC*CR/(BW)$$

MC represents the heavy metal concentration (μg/g wet weight) of the collected *Ipomoea aquatica*. The body weight (BW; kg) for adult Filipino male is 61.3 kg while for Filipino female is 54.3 kg [24]. The consumption rate (CR; g/person/day) for *Ipomoea aquatica* vegetable is 6 g/capita/day, following the report of Woolfe [38]. As for human risk assessment of Cd, Cu, Ni, Pb and Zn, the Target Hazard Quotient (THQ) was utilized. According to Bogdanovic et al. [25], a THQ value of > 1.0 means that the daily intake of *Ipomoea aquatica* would likely result in negative health effects during a lifetime of the consumer. The equation of THQ calculation was described as follows:

$$THQ = EDI/RfD$$

To obtain target hazard quotient (THQ), estimated daily intake EDI was divided by oral reference dosage (Rfd). A reference dose (of mg/Kg) was determined for Cd (0.001), Cu (0.04), Ni (0.01), Pb (0.004) and Zn (0.03) based on the Regional Screening Levels of the US EPA [26].

3. Results and Discussion

3.1. Soil Characteristics of the Experimental Area

The soil at Barangay Mangan Banga, Aklan, is located at coordinates 11.631781°N and 122.54795°E with an elevation of four meters above mean sea level (masl). The soil is classified under Alimodian clay loam, the most dominant soil type in the province. The soil terrain is flat, well-drained, but shows a moderate degree of soil compaction due to cattle grazing. Vegetation includes grasses and shrubs as well as high-value crops like papaya (*Carica papaya*), eggplant (*Solanum melongena*), calamansi *(Citrofortunella microcarpa)*, and soursop *(Annona muricata)*.

Surface soil horizon (0-35 cm) has dark gray color, 7.5 YR 4/1; clay loam texture; fine to medium granular structure; moderately sticky and plastic, common medium, fine to medium pores; common large, medium, fine roots; smooth boundary. Subsoil (35-126 cm) has a dark grayish brown color, 10 YR 4/2; clay texture; medium to fine subangular blocky structure; slightly sticky, slightly plastic when wet, hard when dry; very few very fine to large pores; very few very fine roots, common coarse roots; clear smooth boundary. Lower subsoil (126-300 cm) has strong brown color, 7.5 YR 4/6; clay loam; has fine to medium, angular blocky structure; sticky and plastic, harsh when dry; has the presence of very few, very fine, and large roots; presence very few very fine pores. The surface soil has low inherent fertility, which contains 0.07 % N, 0.0069% P, and 0.008 % K. The amount of OM was 1.48%, and the pH level was 5.7 classified as moderately acidic. The soil's heavy metals content (mg/kg) was in the order of copper-8.56, Zinc 8.14, Lead- 2.83, Arsenic - 0.05, Cadmium - 0.3, Nickel - 0.12, Mercury <0.001, and Selenium <0.001.

3.2. Chemical Properties and Heavy Metals of Stabilized Biosolids

The initial data on the chemical properties of the stabilized biosolids from the wastewater treatment facility in Boracay Island is presented in Table 1. It indicated that stabilization significantly affected other chemical properties such as pH, electrical conductivity, and phosphorus and potassium levels. No significant differences were noted in the amount of organic matter, total organic carbon, nitrogen, and C/N ratio. Vermistabilization has the best potential compared to other techniques in stabilizing biosolids (Table 1). Although the vermistabilized biosolids obtained significantly lower Cd, Cu, and Zn levels than the other treatments, they contained higher nickel and lead levels. Most stabilization processes, particularly natural stabilization, photo stabilization, effective microorganism stabilization, and indigenous microorganism stabilization, have significantly higher levels of Cd and Cu. Generally, the obtained levels of heavy metals were within the permissible levels of DAO 2013-22 [27] (Table 2).

Table 1. Summary of the chemical parameters of stabilized biosolids from the wastewater Treatment Plant (WWTP) in Boracay Island

TREATMENT	pH	EC dS/m	OM %	TOC %	N %	C/N Ratio	P %	K %
Natural Stabilization	6.00	1.09	19.57	11.75	0.98	11.70	0.03	0.02
Photo Stabilization	6.60	1.22	20.03	11.85	1.00	11.07	0.04	0.02
Effective Microorganism Stabilization	6.23	1.43	16.20	9.50	0.81	11.63	0.05	0.03
Indigenous Microorganisms Stabilization	6.13	1.57	16.10	9.70	0.81	12.94	0.05	0.04
Vermistabilization	7.00	2.43	17.65	10.33	0.88	11.78	0.06	0.23

Table 2. Summary of the heavy metals content of stabilized biosolids from the WWTP in Boracay Island

TREATMENT	HEAVY METALS (µg/g)				
	Cd	Cu	Ni	Pb	Zn
Natural Stabilization	0.04	0.92	0.01	0.43	1.04
Photo Stabilization	0.04	0.87	0.01	0.45	0.91
Effective Microorganism Stabilization	0.04	0.83	0.01	0.42	0.69
Indigenous Microorganisms Stabilization	0.04	0.50	0.20	0.31	0.34
Vermistabilization	0.02	0.32	0.26	0.68	0.44

Note: BAFS (2016) standard for Cd-5.0, Pb-50; FPA (2005) standard for Cu-5.0, Ni-5.0, Zn-333 (maximum allowable level of heavy metals for solid/compost/soil conditioner mg/kg dry wt)

3.3. The Chemical Characteristics of Soils Applied with Stabilized Biosolids

3.3.1. Soil pH

Soil pH is a measure of the acidity or alkalinity in the soil. It is also called “soil reaction.” The use of biosolids has significantly affected the pH levels of the soil. As obtained in the study, the initial pH of the soil was 5.7. After the study, soils applied with indigenous microorganism-stabilized biosolids had a pH value of 6.8 (neutral). This pH did not differ significantly from the pH of the soils applied with other stabilization methods such as vermistabilization (6.6-neutral), natural method (6.1-slightly acidic), photocatalyst (6.5-slightly acidic), and effective microorganism (6.3-slightly acidic). Soil without biosolids or the control plots had a pH of 5.7, indicating a more acidic soil environment (Table 3). The decrease in the acidity of the soil-applied with stabilized biosolids could be attributed to the presence of $CaCO_3$ used in treating biosolids in the WWTP. In addition, in soil systems, the pH is affected by mineralogy, climate, and weathering. Management of soils often alters the natural pH because of acid-forming nitrogen fertilizers or the removal of bases (potassium, calcium, and magnesium). Soils with sulfur-forming minerals can produce very acidic soil conditions when exposed to air [28].

3.3.2. Electrical Conductivity (EC)

Soil electrical conductivity (EC) is a measure of the amount of salts in soil (salinity of soil). In this study, the value of soil EC was not affected by applying various biosolids stabilization processes. The electrical conductivity of the soils ranged from 0.003 to 0.009 dS/m (Table 3). Based on the salinity threshold of the USDA [29], soil electrical conductivity values of 0 to 1.4 dS/m are not saline, and this does not impact most crops and soil microbial processes. Soils applied with biosolids with lower electrical conductivity values have more extended periods to develop soil alkalinity problems, which could gradually affect the soil quality. The electrical conductivity of the soil is affected by cropping, irrigation, land use, fertilizer application, manure, and compost.

3.3.3. Organic Matter Content

The organic matter content of the soil planted with *Ipomoea aquatica* was significantly affected by the applied stabilized biosolids (Table 3). Soils applied with indigenous microorganism-stabilized and vermi-stabilized biosolids have 2.33% and 2.27% organic matter, respectively. Soil without biosolids (NF) has 1.5% organic matter content. General observation showed that applying biosolids stabilized using microorganisms and earthworms resulted in a slight build-up of organic matter in the soil. Organic matter content is an essential characteristic of biosolids. As concluded by Torri et al [30], the application of biosolids can increase the soil organic matter (SOM) and improve desirable soil qualities.

Table 3. Summary of the chemical parameters of soils applied with stabilized biosolids from the WWTP in Boracay Island

TREATMENT	pH*	EC (dS/m) ^{ns}	OM (%) *	N (%) *	P (%) ^{ns}	K (%) ^{ns}
Natural Field Condition	5.77^{c}	0.003	1.50^{b}	0.08^{b}	0.001	0.009
Natural Stabilization	6.13^{abc}	0.005	1.50^{b}	0.08^{b}	0.002	0.010
Photo Stabilization	6.50^{abc}	0.004	1.73^{ab}	0.09^{ab}	0.002	0.014
Effective Microorganism Stabilization	6.30^{abc}	0.008	1.90^{a}	0.10^{a}	0.004	0.013
Indigenous Microorganisms Stabilization	6.83^{a}	0.009	2.33^{a}	0.12^{a}	0.003	0.098
Vermistabilization	6.60^{ab}	0.004	2.27^{a}	0.11^{a}	0.002	0.014
Inorganic Fertilizer Recommended Rate	6.00^{bc}	0.004	1.87^{a}	0.09^{ab}	0.003	0.010
CV (%)	4.46	7.74	17.63	17.60	8.42	5.80

**Means with the same superscript are not significantly different at $p<0.05$, LSD test; ^{ns} Not significant*

3.3.4. Nitrogen, Phosphorus, and Potassium Contents of the Soil

The application of stabilized biosolids significantly affected the nitrogen contents of the soils (Table 3). Soils applied with indigenous, vermicomposting, and effective microorganism-stabilized processes obtained the highest level of N with 0.12, 0.11, and 0.10%, respectively. The soil's nitrogen content with photostabilized biosolids was comparable with the N of soil-applied with inorganic fertilizer. The soil without fertilizer had an indigenous N content of 0.08%. The excellent performance of IMOs has been reviewed by Kumar and Gopal [8], wherein IMOs were found to be effective in biodegradation, nitrogen fixation, and soil fertility improvement. On the other hand, effective microorganisms contain groups of environment-friendly organisms, such as bacteria and beneficial fungi, which work with other microorganisms and organic matter to enhance the production of plant nutrients, enzymes, and other bioactive substances.

The phosphorus content of the soil was not affected by the application of the variously prepared biosolids (Table 3). It can be noted, however, that soils with indigenous microorganisms and effective microorganism-stabilized biosolids had considerable amounts of P. The beneficial effects of indigenous and effective organisms such as *Aspergillus, Penicillium, Enterobacteria,* and *Pseudomonaceae* were recognized as the most potent phosphate solubilizers that could be responsible for the presence of P in the soil [31]. The amount of K in the soil did not differ significantly with the application of biosolids compared to the unfertilized plot (Table 3). Conversely, soils applied with biosolids stabilized through microbial actions, and earthworms had more available K than the unfertilized plot. Some biosolids had limited amounts of K [32]. Hence, its application in the soil would not result in the build-up of K. In addition, once applied in the soil, K is exposed to different losses, particularly through leaching, fixation, and plant uptake. These losses can be related to the general characteristics of the agricultural ecosystem, cropping or grazing, tillage management, interactions with other nutrients such as nitrogen and specific features such as soil mineralogy, texture, initial soil K status, sources of K applied (organic, inorganic) and rates and timing of fertilizer applications [33].

3.4. Nitrogen, Phosphorus, and Potassium Contents of Soil

The presence of heavy metals in biosolids is the primary concern that limits their application in agricultural soil. Since heavy metals can accumulate in hazardous levels in the soils after repeated application, they can migrate to the ecosystem and transfer along the food chain [34]. Information on their mobility, bioavailability, and ecotoxicity to plants is essential. Table 4 shows the summary of heavy metals assessed in this study, such as cadmium (Cd), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn).

The amount of Cd in the soil applied with stabilized biosolids was 0.01 (µg/g). The value is below the standard for soil set by the Department of Environment and Natural Resources, which is 0.03 (µg/g) [27]. Copper levels in soils treated with stabilized biosolids differed significantly. The highest Cu level of 0.96 µg/g was obtained in soils with natural and EMS-stabilized biosolids. This amount of Cu did not differ considerably from the levels of Cu in other soils applied with biosolids stabilization processes. Soils applied with inorganic fertilizer had the lowest Cu levels of 0.84 µg/g.

The level of nickel obtained in the soils was 0.009 µg/g. The value can be considered very low based on the DAO-BAFS standards of 5.0 mg/L. The lead content of the soil did not vary significantly among treatments. The values of the Pb levels ranged from 0.14 to 0.2 µg/g. Based on the DAO 2012-13 standards, the value was below the threshold levels for Pb, which is 1 µg/g. Zinc levels in the soil applied with stabilized biosolids did not vary significantly. However, soils applied with EM and IMO stabilized biosolids had more Zn with 1.02 and 1.04 µg/g Zn, respectively. A low Zn level was obtained in soils applied with inorganic fertilizer.

Table 4. Summary of the heavy metal content of soils applied with stabilized biosolids from the WWTP in Boracay Island

TREATMENT	HEAVY METALS (µg/g)				
	Cd ns	Cu*	Ni ns	Pb ns	Zn ns
Natural Field Condition	0.01	0.937ab	0.009	0.200	0.851
Natural Stabilization	0.01	0.963a	0.009	0.190	0.967
Photo Stabilization	0.01	0.938ab	0.009	0.140	0.966
Effective Microorganism Stabilization	0.01	0.960a	0.009	0.170	1.017
Indigenous Microorganisms Stabilization	0.01	0.932ab	0.009	0.137	1.040
Vermistabilization	0.01	0.878ab	0.009	0.140	0.915
Inorganic Fertilizer Recommended Rate	0.01	0.837b	0.009	0.140	0.764
CV (%)	-	4.50	-	16.44	13.03

**Means with the same superscript are not significantly different at $p<0.05$, LSD test; nsNot significant*

Table 5. Summary of the growth and yield of *Ipomoea aquatica* applied with stabilized biosolids from the WWTP in Boracay Island

TREATMENT	PLANT HEIGHT (cm)		TILLER NUMBER*	YIELD (Mg/ha) *
	15 DAS*	45 DAS*		
Natural Field Condition	25.02bc	44.58b	3.07b	10.32b
Natural Stabilization	28.26abc	48.35ab	3.33ab	16.77ab
Photo Stabilization	28.68abc	52.93ab	4.03ab	18.61ab
Effective Microorganism Stabilization	26.66abc	55.72ab	3.87ab	25.35a
Indigenous Microorganisms Stabilization	30.06ab	60.77ab	4.53a	26.05a
Vermistabilization	31.66a	63.62a	4.00ab	27.06a
Inorganic Fertilizer Recommended Rate	22.60c	55.08ab	3.03b	20.40ab
CV (%)	8.46	11.02	12.07	23.12

**Means with the same superscript are not significantly different at $p<0.05$, LSD test*

Availability of heavy metals in unfertilized soils (NS) and soils applied with inorganic fertilizer were in the order of Cd<Ni<Pb<Zn<Cu, while in the soils applied with naturally stabilized (NS), photostabilized (PS), indigenous microorganism stabilized (IMS), effective microorganism stabilized (EMS) and vermistabilized (VS) biosolids, the availability was in the order of Cd<Ni<Pb<Cu and Zn. The dilution effect and leaching processes in the soils resulted in the decreased concentrations of heavy metals Cd, Ni, Pb. On the other hand, the high inherent value of Cu and Zn increased their concentrations in the soil.

3.5. The Yield of *Ipomoea aquatica*

Ipomoea aquatica plants applied with biosolids stabilized by indigenous microorganisms, effective microorganism and vermicomposting produced the highest yield of 25.35, 26.05 and 27.06 Mg ha^{-1}, respectively (Table 5). The results did not differ significantly with naturally stabilized, photostabilized and those applied with inorganic fertilizer. Controlled plants had the lowest yield of 10.32 Mg ha^{-1}. The beneficial effects of biosolids and the consortium of earthworm and microorganisms resulted in the favorable growth and yield of *Ipomoea aquatica.*

Vermicomposting and microbial degradation of indigenous and effective microorganisms release enzymes like amylase, lipase, cellulose, and chitinase, which break down organic matter, releasing essential nutrients and making them bioavailable for plant growth. The enhanced growth in the plant may also be due to improved soil health and the physicochemical properties of soil. The crop stands of *Ipomoea aquatica* applied with different biosolids are shown in Figure 1.

Figure 1. Crop stand of *Ipomoea aquatica* applied with different types of stabilized biosolids from the Wastewater Treatment Facility in Boracay Island

3.6. Heavy Metal Contents of Plant Applied with Stabilized Biosolids

After accumulating in the soil, some metals are transferred to the food chain, which can cause serious health hazards to humans, plants, and animals [35]. To evaluate the bioaccumulation of heavy metals from the biosolids-amended soil, water spinach, or *kangkong* (*Ipomoea aquatica*) was used due to its metal hyperaccumulating properties.

The concentration of cadmium (Cd) uptake of *Ipomoea aquatica* did not vary significantly in soils with or without biosolids (Table 6). Plants grown in IMO-stabilized, vermistabilzed and inorganically fertilized soils had 0.009 µg/g Cd, whereas those plants grown under EM-stabilized and photostabilized biosolids had 0.010 µg/g Cd. The Cu uptake of *Ipomoea aquatica* did not vary significantly among soils applied with different methods of biosolids stabilization. The Cu content in plants ranged from 0.24 µg/g obtained from inorganically fertilized plants to 0.51 µg/g from unfertilized soil. The values were within the limit set by the WHO for Cu, which is 3.0 µg/g [36]. Nickel concentration of the plants was not significantly affected by the application of the different biosolids stabilization processes. The permissible limits and maximum allowable threshold for Ni permitted by WHO is 0.05 µg/g [37].

Uptake of Pb in *Ipomoea aquatica* plant did not vary significantly among the various treatments. Plant Pb content ranged from 0.016 µg/g in unfertilized (NS) to 0.040 µg/g in inorganically fertilized (IF) soil. WHO's threshold limit in Pb levels for edible plant tissue is 0.43 µg/g, indicating that the plant can be considered safe for human consumption [36]. There were no significant differences in the amount of Zn in the plants applied with various types of stabilized biosolids. Plant Zn levels ranged from 0.344 µg/g (NF and IF) to 0.550 µg/g (IMS). The values were very low, based on the limit of 47.4 µg/g set by the WHO [36]. Generally, the availability of heavy metals present in the plant applied with different stabilized biosolids was inoder of Cd<Pb<Cu<Ni<Zn.

3.7. Human Health Risk Assessment of Heavy Metals Exposure through Ipomoea aquatica Consumption Planted in Biosolids-Amended Soils

Humans are chemically exposed to food coming from a chemically complex environment. Human health risk assessment helps experts assess the overall situation and determine what advice or actions should be taken to ensure that human health is protected. Past, current, or future exposures to chemicals in the air, soil, water, food, consumer products, or other materials may be assessed [23].

In this study, the human health risk assessment through consumption *Ipomoea aquatica* growing in a medium that contains trace elements was determined by getting the average daily intake (ADI) of adult male and female Filipino consumers. Average daily intake was obtained using the formula ADI = metal wet concentration x consumption rate of *Ipomoea aquatica*/bodyweight of the male and female consumers. According to Woolfe [38], the per capita consumption of *Ipomoea aquatica* among Filipinos is 6.03 g/day. The average body weight for the adult male is 61.3 kg, and for the female is 54.3 kg based on the Food and Nutrition Research Institute (2013) data. The target hazard quotient (THQ) was obtained by dividing the ADI with the oral reference dose (ORD) for each metal according to the Regional Screening Levels set by the United State Environment Protection Agency [39]. The oral reference dose (mg/kg/day) for the various heavy metals were Cd-0.001, Cu-0.04, Ni-0.02, Pb-0.004 and Zn-0.3. The computed data on THQ for female and male consumers (Tables 7 and 8) using the reference values indicated that the THQ values for females ranged from 1×10^{-4} to 1.4×10^{-4} for Cd, 3×10^{-5} to 4×10^{-5} for Cu, 1×10^{-4} to 1.5×10^{-4} for Ni, 4×10^{-5} to 1.1×10^{-4} for Pb and 3×10^{-6} to 4×10^{-5} for Zn. Conversely, the THQ values for men ranged from 9×10^{-5} to 1.3×10^{-4} for both Cd and Ni, 2×10^{-5} to 3×10^{-5} for Cu, 4×10^{-5} to 1.0×10^{-4} for Pb and 2×10^{-6} to 3×10^{-5} for Zn. All the THQ values of the heavy metals evaluated in association with *Ipomoea aquatica* consumption, were all less than one (<1). As implied, if the THQ value is more than one (>1), it shows that the level of exposure is higher than the oral reference dose (ORD), which assumes that daily exposure at this level is likely to cause harmful health effects during the lifetime of a human population. In this study, all the obtained THQ values were <1, indicating no apparent risk in the consumers lifetime [40].

Table 6. Summary of the heavy metal contents in plants applied with stabilized biosolids from the WWTP in Boracay Island

TREATMENT	HEAVY METALS (µg/g)				
	Cd[ns]	Cu[ns]	Ni[ns]	Pb[ns]	Zn[ns]
Natural Field Condition	0.010	0.51	0.019	0.016	0.344
Natural Stabilization	0.013	0.33	0.011	0.019	0.435
Photo Stabilization	0.010	0.41	0.016	0.023	0.337
Effective Microorganism Stabilization	0.010	0.32	0.016	0.026	0.390
Indigenous Microorganisms Stabilization	0.009	0.41	0.010	0.033	0.550
Vermistabilization	0.009	0.24	0.019	0.036	0.339
Inorganic Fertilizer Recommended Rate	0.009	0.34	0.019	0.040	0.344
CV (%)	23.14	26.43	12.88	22.32	27.84

[ns]Not significant

Table 7. Target hazard quotient (THQ) values for heavy metals in *Ipomoea aquatica* applied with stabilized biosolids among female consumers

TREATMENT	THQ				
	Cd	Cu	Ni	Pb	Zn
Natural Field Condition	1.1E-04	3.0E-05	1.2E-04	4.0E-05	3.0E-06
Natural Stabilization	1.4E-04	4.0E-05	1.5E-04	5.0E-05	4.0E-06
Photo Stabilization	1.1E-04	3.0E-05	1.2E-04	6.0E-05	3.0E-06
Effective Microorganism Stabilization	1.1E-04	3.0E-05	1.1E-04	7.0E-05	3.0E-06
Indigenous Microorganisms Stabilization	1.0E-04	4.0E-05	1.4 E-04	9.0E-05	4.0E-06
Vermistabilization	1.0E-04	3.0E-05	1.0 E-04	1.0E-04	3.0E-06
Inorganic Fertilizer Recommended Rate	1.0E-04	3.0E-05	1.0 E-04	1.1E-04	3.0E-06

A THQ value of < 1 indicates no apparent health risk (Dee et al., 2019).

Table 8. Target hazard quotient (THQ) values for heavy metals in *Ipomoea aquatica* applied with stabilized biosolids among male consumers

TREATMENT	THQ				
	Cd	Cu	Ni	Pb	Zn
Natural Field Condition	1.0E-04	3.0E-05	1.1E-04	4.0E-05	3.0E-06
Natural Stabilization	1.3E-04	3.0E-05	1.3E-04	5.0E-05	3.0E-06
Photo Stabilization	1.0E-04	3.0E-05	1.1E-04	6.0E-05	3.0E-06
Effective Microorganism Stabilization	1.0E-04	3.0E-05	1.0E-04	6.0E-05	3.0E-06
Indigenous Microorganisms Stabilization	9.0E-05	3.0E-05	1.3E-04	8.0E-05	3.0E-06
Vermistabilization	9.0E-05	2.0E-05	9.0E-05	9.0E-05	2.0E-06
Inorganic Fertilizer Recommended Rate	9.0E-05	2.0E-05	9.0E-05	1.0E-04	2.0E-06

A THQ value of < 1 indicates no apparent health risk (Dee et al., 2019).

4. Conclusions

The effects of stabilized biosolids from the Sewage Treatment Plant in Boracay Island, Philippines on selected soil properties, heavy metals, and target hazard quotients associated with *Ipomoea aquatica* consumption were evaluated. Applying stabilized biosolids in soils has no significant effects on electrical conductivity, phosphorus, and potassium levels but significantly affects the pH, organic matter, and nitrogen content. The amount of heavy metals, particularly cadmium, nickel, lead, and zinc, generally decreased in the soils except for Cu. Plant yield was generally enhanced by applying stabilized biosolids comparable to inorganic fertilizer. The target hazard quotient (THQ) levels for all the heavy metals evaluated with *Ipomoea aquatica* consumption by female and male adult Filipinos were all less than one (<1), indicating no obvious risk on health effects on the consumers' lifetime.

Despite the desirable results, to further ensure the safe use of biosolids in the agricultural ecosystem and to avoid backlash due to the buildup of heavy metals, site-specific studies on the potential use of biosolids in crops should be considered given the dynamic properties of soils affecting the behavior of both the inherent and added heavy metal, as well as nutrient components. Routine analyses of nutrients, heavy metal translocation, and uptake of crops may be included in the crop production activities involving biosolids to guarantee the safety of consumers and foster soil environment protection and quality.

Conflict of Interest

The author declares no conflict of interest.

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