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Vetiver (*Vetiveria Zizanioides*) As Phytoremediator on Chromium and Nickel Uptake in Lowland Rice Soils Affected by Mining Activities

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Abstract
The use of vetiver (Vetiveria zizanioides) is one of a few plant species meeting all the criteria required for phytoremediation. However, very limited studies have been done to use this plant as a phytoremediator in lowland rice soils. The study was conducted to determine the effect of phytoremediation using different time durations of vetiver grown for 3 and 8 months in the field and to evaluate the accumulation and translocation of Cr and Ni at various growth stages of the crop after planted with 3 months of vetiver. The field experimental set-up was conducted for two cropping seasons. Results revealed that vetiver had a Bio-accumulation factor of 0.97 for Cr and 1.07 for Ni after 3 months of growth and 1.48 for Cr and 1.38 for Ni after 8 months, indicating a higher concentration of Cr and Ni in the plant than in the soil. The translocation factors at both 3 and 8 months were below 0.2 mg kg -1 suggesting that vetiver accumulates Cr and Ni in the roots and very small amount is translocated to the shoots, making the plant safe for forage purposes. The Cr and Ni content in rice grains after planting the area with 3 months vetiver, were efficiently reduced to below detection limits.

Keywords: *bio-accumulation, translocation, phytoremediation, soil pollutants.*

Introduction

The Philippines has an estimated USD840 billion worth of untapped mineral resources, according to the Mines and Geosciences Bureau of the Philippines (2015). Large-scale mining is destructive, as it uses the open-pit method which entails clearing thousands of hectares of rainforests and agricultural lands, making deep excavations to extract minerals, using of toxic heavy metals and chemicals to process mineral ores, and consuming millions of liters of water. All of these negatively impact the lives of the Filipino citizens with the grave disregard for their right to health, life, food security, livelihood, and a clean environment.

Mining companies contribute to the massive siltation of the rivers, poisoning the waterways and agricultural fields with toxic chemicals and rendering communities more vulnerable to flooding. At the same time, local communities affected by mining bewail the loss of their former livelihood in fishing, agriculture and forestry, (Salamat, 2013). Heavy metals from mining operations find their way into streams and rivers and percolate into ground water. Contamination of a high level may cause destruction of farm crops with resultant injuries to humans (Iddrisu & Akabzaa, 1996).

Agricultural soils may be contaminated by heavy metals through practices such as irrigation and use of heavy metals containing agrochemicals such as pesticides, herbicides and fertilizers. The greatest risk of contamination has been reported in soils around mining operations (Simon et. al., 2016). Plants have a natural tendency to absorb toxic substances including heavy metals which are eventually transferred from the mining industry. Their high solubility in the aquatic environment is the main reason for the decrease in the production of food grains and vegetables as well as the adverse effect on the health of human beings.

Soil rehabilitation using phytoremediator like vetiver is a practical and low-cost measure that can be used to inhibit the uptake of heavy metals by crops grown on contaminated agricultural soils. Danhl et al., (2009) showed that vetiver grass can produce high biomass (>100t ha-1 year-1) and can highly tolerate extreme climatic variation. Hence, it can accumulate heavy metals, particularly lead, chromium, nickel (shoot 0.4% and root 1%), and zinc (shoot and root 1%). The majority of heavy metals are accumulated in its roots, hence, it is suitable for phytostabilization and for phytoextraction. Furthermore, it was found to be highly tolerant to an extremely adverse condition. Thus, it can be used for rehabilitation of mine tailings, garbage landfills, and industrial waste dumps that are often extremely acidic or alkaline, and soils high in heavy metals (Roongtanakiat et al., 2009). All these special characteristics make vetiver a choice plant for the phytoremediation of heavy metals and organic wastes since vetiver grass is more efficient in absorbing certain heavy metals and chemicals due to the capacity of its root system to reach greater depths and widths.

Based on the identified problems in Santa Cruz, Zambales, there is an urgent need to determine cost-effective techniques for mitigating heavy metals in a contaminated rice field. Since accumulation of heavy metals like Cr and Ni, particularly in agricultural soil brings disorder of soil function which, in turn, affects crop growth. The heavy metals can be transferred to crops therefore posing a risk to human health. The study was conducted based on the hypothesis that the accumulation of chromium (Cr), and nickel (Ni) in the rice field of Santa Cruz, Zambales has greatly affected rice production as a result of the mining industry and the removal of these metals will result to improve growth and yield of lowland rice. The results of this study can serve as a basis for mitigating heavy metal pollution in the affected area. Thus, it was conducted to determine the effect of (phytoremediation) vetiver grown for 3 and 8 months in the field on the accumulation and translocation of Cr and Ni by lowland rice grown in contaminated soil and to evaluate its effect on the translocation and accumulation of Cr and Ni in rice at various growth stages of the crop after planted with 3 months vetiver.

Methodology

The Study Area

Ocular survey was conducted in the vicinity of a mining area in Santa Cruz, Zambales. The basic criterion for the selection of the study site was the presence of an irrigated rice field. The field experiment was conducted from June 2017 to February 2018 in Barangay Lomboy, Sta. Cruz, Zambales. The geographical location of the study area is 15o46’38.1”N latitude and 119 o56’14.1”E longitude with elevation of 11.8 m above sea level (Figure 1).

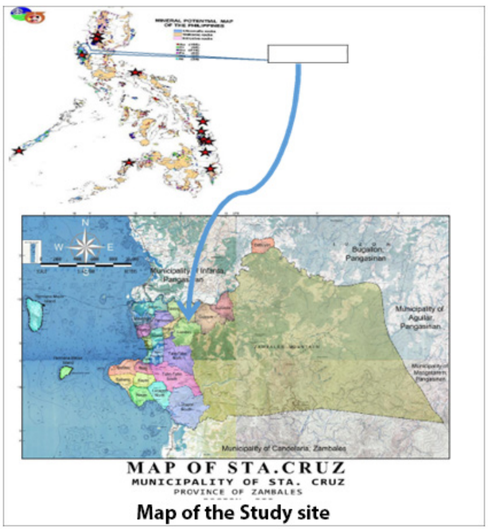

Figure 1: Map showing the location of the study site.

Cultural and Management Practices

For the first cropping season (2017 WS), the area was planted with a phytoremediator (vetiver). The planting material was used from the Department of Agronomy and Agroforestry, UP Los Banos with a length of 25 cm. The experimental area was divided into 20 plots, each having a dimension of 4m x 5m and spaced at 0.50m from each other. During the second cropping season (2018 DS) the area previously planted with vetiver plant was divided into two (2) sub-blocks. One sub-block was grown with rice using the farmer’s practice of fertilization while the other sub-block remained planted with vetiver. The experimental field was tilled before planting with a moldboard plow, and then two passes with a leveling disk were done. The first one was done soon after the moldboard plow tillage and the second one shortly before transplanting. Bunds and drainage canals were constructed around the experimental field. Final leveling was done after puddling the soil. Sprouted rice seeds at 60 kg ha^{-1} NSIC Rc160 (Tubigan 14) seed variety was sown on a nursery seedbed to raise the seedlings. Fertilizers were applied based on the recommended rate of the Rice Crop Manager (RCM). 129-36-46 kg N P_2O_5 K_2O ha^{-1} was applied. Basal application of 5.07 bags (253.33 kg) of 14-14-14 (complete fertilizer) ha-1 before transplanting and second application at 21-25 DAT with a rate of 1.93 bags (96.67 kg) of 46-0-0 (urea) ha-1 and third fertilizer application at 35-39 DAT with a rate of 2.13 bags (106.67 kg) of 46-0-0 (urea) ha^{-1} and 0.33 bag (16.67 kg) of 0-0-60 (muriate of potash) ha^{-1}. Seventeen to twenty-day-old seedlings were transplanted in the field on November 2017 (dry season) at 0.20m x 0.20m planting distance with 2-3rice seedlings per hill. Missing hills were replanted during the first two weeks after transplanting to ensure uniform crop stand. All plots were kept weed-free during the growing season, particularly before each fertilizer application. Pests and insects were controlled according to the best management standard for irrigated rice production at the farm. The crop was supplemented with irrigation water if required. Excessive water and water deficit that could affect the growth and yield of the crop were avoided. Intermittent or controlled irrigation system was employed. Water depth of 3–5 cm was maintained at every irrigation time from early tillering until 1-2 weeks before crop maturity or harvest.

Harvesting was carried out when the crops reached physiological maturity (85% of the spikelets in a panicle were yellow) by cutting the culms close to the ground and threshing immediately. Yield samples were taken from 5.0 m^2 (2.5 m x 2.0 m) sampling area. Samples were cleaned and sundried then oven dried until 14% moisture content (MC) was attained. Soil samples were collected before and after harvesting for two (2) cropping seasons. Before planting, about one kilogram of composite soil sample was collected from the top 20cm layer using soil auger and was placed in a properly labeled plastic container. After planting, soil samples (composite) were collected per plots from the top 20 cm layer using soil auger and were placed in a properly labeled plastic container. Two (2) metal ions were considered to be investigated in this study namely: Chromium (Cr) and Nickel (Ni).

Data Gathered

Soil sampling was conducted before and after planting and samples were analyzed for various soil properties, as follows: Soil texture (Hydrometer Method), pH in water (Potentiometric Method), organic matter (Walkley-Black Method), total N (Kjeldahl Method), available P (Bray No. 2 Method), exchangeable K (Flame Spectrophotometer Method), and cation exchange capacity (NH4OAc Method). Heavy metal (Total Cr and Ni) and (Available Cr and Ni) concentrations in the soil were analyzed using acid digestion on Microwave plasma-Atomic Emission Spectrometer Method (ICP-AES). All the analyses were conducted at the Regional Soils Laboratory-Department of Agriculture in Caraga Region. The available Cr and Ni concentrations in the soil were represented in the availability of these metals in the field for plant absorption. Also, the analytical results served as a guide in identifying constraints for agricultural crop production in the area, and in formulating recommendations for rehabilitation/mitigation.

Agronomic parameters were determined such as plant height at (14DAT) vegetative stage, (50DAT) panicle initiation stage, and 80-85% maturity. Plant height was measured, and at the same time, the tillers were counted. Panicle per hill, spikelets per panicle, percent filled spikelets, weight of 1000 grains, harvest index, and grain yield. Return on investment was also computed.

Plant tissue samples were collected at 14 DAT (vegetative stage), 50 DAT (panicle initiation stage), and maturity. Samples were cleaned from dust and soil particles or any contamination, then oven-dried at 700 C to minimize loss of soluble constituents and to avoid thermal decomposition of the material. Oven-dried tissue samples were ground and placed in a properly labeled coin envelope and stored in a desiccator. The root and straw of rice from 14 DAT (vegetative stage) and 50 DAT (panicle initiation stage) were analyzed separately for Cr and Ni, while at harvest, root, straw and rice grain were segregated for N, P, K, Cr, and Ni analyses. However, for vetiver plants only root and shoot were determined for N, P, K, Cr, and Ni. Analyses were determined using Kjeldahl N analyzer, Spectrophotometer and acid digestion using Microwave plasma- Atomic Emission Spectrometer Method (ICP-AES) at the Regional Soils Laboratory-Department of Agriculture in Caraga Region. Bioaccumulation and translocation factor were also calculated based on the concentration of Cr and Ni in the soil and plants.

Results and Discussion

General Description of the Study Area

Field experiments were conducted during 2017 WS (June – September) and 2018 DS (November 2017 – February 2018) in Barangay Lomboy, Sta. Cruz, Zambales. Sta. Cruz, is the first-class municipality of Zambales. It has a rich arable land that is conducive for farming, particularly rice. However, the area is suffering from devastating effects of mining industry because of its abundant deposit of chromite and nickel minerals (Environmental Justice Atlas, 2015) causing a major source of farmland heavy metal contamination together with other inorganic materials deposited by surface run-off and or percolation from higher topographic position in the landscape. Besides, heavy laterite siltation of river systems, and farmlands were observed in the area which severely reduced the palay production.

Soil Characteristics

The soil type in the study area is Bani silty clay with initial soil characteristics are presented in Table 1. It appears that, the area has an adverse soil fertility condition as indicated by it's extremely to strongly acidic pH (4.38 to 5.1), low OM (1.52 to 1.76 %), low total N content (0.15 %), low CEC (11.32 cmol kg^{-1}), extremely low available P (2.7 mg kg^{-1}), and low exchangeable K (0.14 cmolc kg^{-1}). The soil also contains very high concentrations of total Cr (4314 to 4520 mg kg^{-1}) and available Cr (1875 to 1925 mg kg^{-1}); and total Ni (2648 to 2783 mg kg^{-1}) and available Ni (1689 to 1725 mg kg^{-1}) that may have resulted from the weathering of parent material composed of ultramafic rocks rich in mineral resources such as Ni, chromite, Fe and Mn (Sevilla, 2003). The improper disposal of mining waste from chromite and nickel mines also resulted in groundwater contamination or seepage in the area. Moreover, the continuous use of irrigation water contaminated with Cr (<0.05 mg kg^{-1}) and Ni (0.019 mg kg^{-1}) has also aggravated the situation. Soil organic matter content was higher on the surface horizon (0-20 cm) compared to the sub-surface horizon (20-40 cm) probably due to its higher amount of moisture, plant litter and temperature received that favor the decomposition process. When these variables are put together, percent organic matter increases (Antonladis et al., 2008). Cation exchange capacity (CEC) was higher on the surface compared to the sub-surface soil. This is attributed by higher OM content on the surface than sub-surface (Table 1). It is usually means that high CEC soils have a greater nutrients and water holding capacity than those with low CEC. It was also observed that the experimental area contains higher concentration of heavy metals such as chromium and nickel. This might be due to the improper disposal of mining waste causing groundwater contamination / seepage from chromite and nickel mines in the area resulting to soil acidity, low organic matter content and CEC. It was also agreed by many other researchers that the availability of heavy metals in soil is associated with several environmental soil factors including pH, soil organic matter content (SOM), and (CEC) cation-exchange capacity (Antonladis et al., 2008). It was also found out that the higher nickel content on the surface than the sub-surface soil might be due to runoff and deposition to the top soil/ surface. However, chromium was present in higher amounts in the subsoil/sub-surface, this might be attributed to the weathering of ultramafic rocks as parent material.

Table 1: Initial soil properties of the experimental area

SOIL PROPERTY	VALUE	RATING / DESCRIPTION*	CRITICAL LEVEL RANGE
Particle size Analysis			
% sand	7.0	-	-
% silt	7.0	-	-
	43.3		
% clay	43.3	-	-
	49.7		
Textural class Soil pH (H_2O,1:1)	Silty clay		
0-20 cm	5.1	Strongly acidic	5.1-5.5
20-40 cm	4.38	Extremely acidic	3.5-4.4
Soil organic matter (%)			
0-20 cm	1.76	Low	1.0-2.0
20-40 cm	1.52	Low	1.0-2.0
Total N (%)	0.15	Low	0.1-0.15
Available P (mg kg^{-1})	2.7	Low	5.0 (acid soils)
			>25.0 (calcareous soils)
Exchangeable K (cmolckg^{-1})	0.14	Low	<0.1
Cation exchange capacity (cmolc kg^{-1})	11.32	Low	<5.0
Total Cr Content (mg kg^{-1})			
0-20 cm	4314	Very high	>240
20-40 cm	4520	Very high	>240
Total Ni Content (mg kg^{-1})			
0-20 cm	2648	Very high	>720
20-40 cm	2783	Very high	>720
Available Cr Content (mg kg^{-1})			
0-20 cm	1875	Very high	50-450
20-40 cm	1925	Very high	50-450
Available Ni Content (mg kg^{-1})			
0-20 cm	1725	Very high	75-150
20-40 cm	1689	Very high	75-150
Soil type	Bani silty clay		

***Source:** DOBERMANN, A AND T. FAIRHURST, 2000. Rice Nutrient Disorders and Nutrient Management. 1st Ed. International Rice Research Institute, Los Banos, Laguna Philippines. 191 pp.

Use of Vetiver in the Phytoremediation of Chromium and Nickel in Contaminated Soils.
Dry Matter Yield, NPK Concentration: and Uptake of Vetiver:

Dry matter yield, NPK concentration and uptake of vetiver used are presented in Table 2. Vetiver grass grew very well in the field. Just after 3 months, it was able to produce a root and shoot biomass of 2,320 and 5,701 kg ha^{-1}, respectively. A notable characteristic of vetiver is the large root dry matter that is produced resulting in a high root to shoot ratio (1:2.5). When grown up to 8 months, root and shoot dry matter yield increased to 3,125 and 7,810 kg ha^{-1}.

NPK concentration in roots and shoots of vetiver grass were relatively high. This result was in agreement with Patra (2011) and Singh et al. (2010) because this type of plant needs higher amounts of nutrients for shoot production. These nutrients are incorporated into their system for their growth and development for higher biomass. Truong and Truong (2011) also reported that vetiver has a very high capacity for uptake of nutrients particularly nitrogen, phosphorus and potassium. Because of the massive and deep roots of vetiver, it was able to take up large amounts of NPK (Figure 2). In 3 months, a total uptake of 35.95 kg N ha^{-1}, 20.54 kg P ha^{-1} and 43.94 kg K ha^{-1} was accumulated in the shoot and 10.44 kg N ha^{-1}, 3.25 kg P ha^{-1} and 6.50 kg K ha^{-1} in the roots. NPK uptake significantly increased after 8 months with 53.89 kg N ha^{-1}, 33.89 kg P ha^{-1} and 72.64 kg K ha^{-1} in the shoot and 17.19 kg N ha^{-1}, 10.31 kg P ha^{-1} and 17.50 kg K ha^{-1}. Hengchaovanich and Nilaweera, (1999) reported that vetiver grass is good for phytoextraction of heavy metals and as well as prevent soil erosion due to its massive, finely structured root system reaching 3-4 m in the first year.

Table 2: Dry matter yield, NPK concentration, and uptake of vetiver grown in the field for 3 and 8 months.

GROWTH DURATION	PLANT PART	DRY MATTER YIELD (kg ha^{-1})	CONCENTRATION (%)			UPTAKE (kg ha^{-1})		
			N	P	K	N	P	K
3 months	Shoot	5707	0.63	0.36	0.77	35.95	20.54	43.94
	Root	2320	0.45	0.14	0.28	10.44	3.25	6.5
8 months	Shoot	7810	0.69	0.43	0.93	53.89	33.58	72.64
	Root	3125	0.55	0.33	0.56	17.19	10.31	17.5

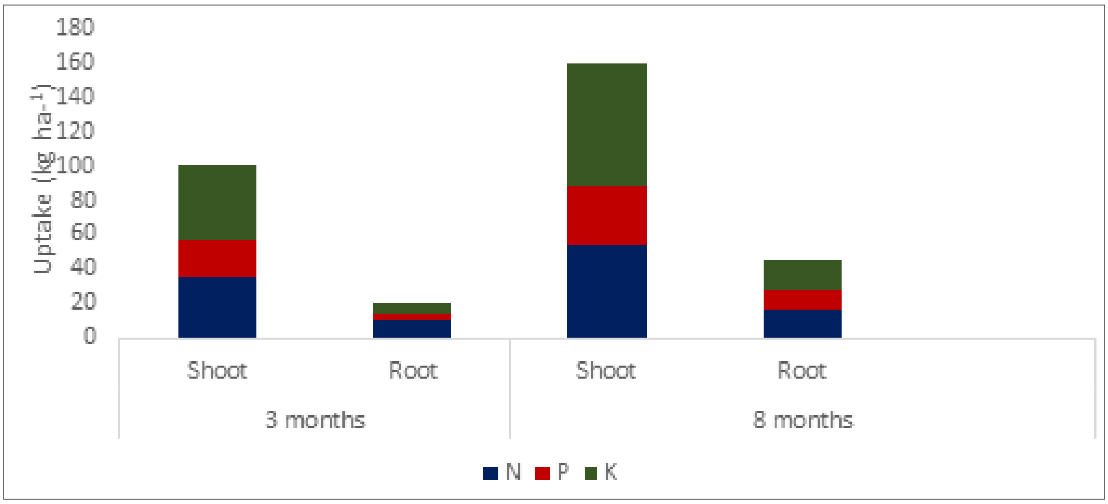

Figure 2: Nutrient partitioning in vetiver grown in the field for 3 and 8 month.

Cr and Ni Concentration and Uptake in Vetiver

A different trend in Cr and Ni concentration and uptake in vetiver are shown in Table 3 and Figure3. More Cr and Ni were accumulated in the vetiver roots compared to the shoots. After growing for 3 months, the vetiver roots were able to accumulate 3.83 kg Cr ha^{-1} and 3.66 kg Ni ha^{-1} while only 0.03 kg Cr ha^{-1} and 0.07 kg Ni ha^{-1} were measured in the shoots. After 8 months, the amount of Cr accumulated in the roots and shoots increased to 6.357 and 0.04 kg Cr ha^{-1}, respectively. For Ni, it only increased to 4.12 kg Ni ha^{-1} and 3.66 kg Ni ha^{-1}. Results implied the importance of vetiver in extracting these metals from the soil and accumulating them in their roots keeping the shoots safe for animal consumption as forage. Due to their extensive rooting system, they can take up considerable amount of heavy metals using ion channels and metal transport proteins in their roots. This type of plant is suited in polluted soils due to its metal accumulating ability coupled with metal tolerance and high shoot biomass (Randloff, et al., 1995; Truong & Baker, 1998; Chen, 2000).

Table 3: Cr and Ni concentration and uptake in vetiver after 3 and 8 months.

CROPPING SEASON	PLANT PART	DRY MATTER YIELD (kg ha^{-1})	CONCENTRATION (mg kg^{-1})		UPTAKE (mg ha^{-1})	
			Cr	Ni	Cr	Ni
2017 WS	Shoot	5706.5	6	12.6	34.24	71.9
	Root	2320	1650	1576	3828	3656.32
2018 DS	Shoot	7810.25	5	5	39.05	39.05
	Root	3125	2102	1319	6568.75	4121.88

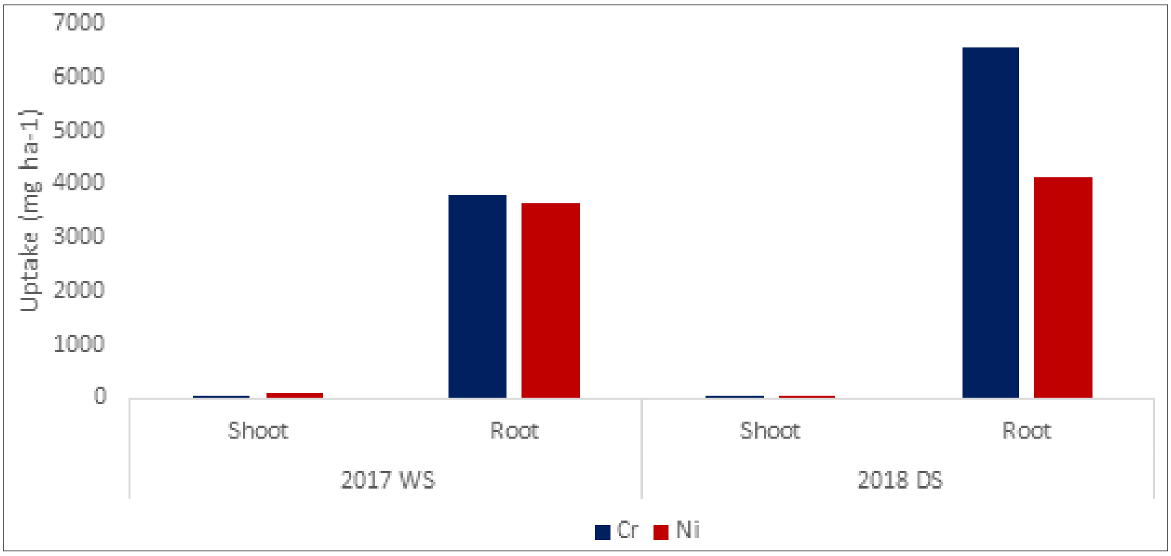

Figure 3: Heavy metal partitioning in vetiver after 3 and 8 months.

Table 4 shows the effect of vetiver plant as phytoremediator on soil pH, organic matter, total and available Cr and Ni. After 3 months, soil pH increased to 6.21 and soil organic matter content to 2.6%. Total Cr and Available Cr were reduced by 186 mg kg^{-1} (4.3%) and 160 mg kg^{-1} (8.53%), respectively. Total and available Ni on the other hand were reduced by 261 mg kg^{-1} (9.86%) and 246 (14.26%) mg kg^{-1}, respectively.

Results indicate that vetiver is very efficient in increasing soil organic matter content due to the unique characteristics of its root system. It has been considered an ideal plant to build up organic matter in poor or degraded soils (Truong 2007). Moreover, vetiver also significantly reduced the amount of total and available Cr and Ni indicating its potential in the phytoremediation of heavy metals.

Table 4: Effect of vetiver plant as phytoremediator on soil parameters:

SOIL PARAMETERS	INITIAL	AFTER 3 MONTHS	INCREASE/DECREASE AFTER 3 MONTHS	AFTER 8 MONTHS	INCREASE/DECREASE AFTER 8 MONTHS
Soil pH (H_2O)	5.10	6.21	1.10	7.12	2.02
Soil organic matter (%)	1.76	2.60	0.84	2.8	1.04
Total Cr (mg kg^{-1})	4314	4128	-186	3821	-307
Total Ni (mg k^{-1})	2648	2387	-261	1850	-537
Available Cr (mg kg^{-1})	1875	1715	-160	1428	-287
Available Ni (mg kg^{-1})	1725	1479	-246	968	-511

Bioaccumulation and Translocation Factors of Vetiver

The bio-accumulation factor (BAF) of vetiver, which is the ratio of the concentration of the heavy metal in the shoot to the soil, is presented in Table 5. Based on the guidelines of Dixit et al. (2001), the BAF of vetiver after 3 months is 0.97 for Cr and 1.07 for Ni which means that vetiver has the capacity to absorb Cr and Ni from the soil and store them in their system, particularly in the roots. Allowing vetiver to grow for 8 months increased BAF to 1.48 for Cr and 1.38 for Ni indicating higher concentration in the plant than in the soil. This further shows the potential of vetiver in mitigating heavy metal pollution in soils.

The translocation factor (TF) of vetiver, which is the ratio of the shoot heavy metal concentration to the root heavy metal concentration, is also presented in Table 5. TF at 3 and 8 months are very low, indicating that vetiver accumulates Cr and Ni in the roots and very small amount of Cr and Ni were translocated to the shoots, making them safe for forage purposes. Higher TF was obtained for Ni as compared to Cr both at 3 and 8 months of growth. These values are below 0.2 mg kg^{-1} which is the prescribed limit given by the Codex Standard and European Union (EU Standards).

Table 5: Bioaccumulation and translocation factors of vetiver after 3 and 8 months of growth

	AFTER 3 MONTHS		AFTER 8 MONTHS		DIFFERENCE	
	Cr	Ni	Cr	Ni	Cr	Ni
Bioaccumulation	0.97	1.07	1.48	1.38	0.51	0.31
Translocation	0.004	0.008	0.002	0.004	-0.002	-0.004

Agronomic Characteristics of Rice Grown after Vetiver

Yield and yield components of rice grown after vetiver are presented in Table 6. Number of tillers and panicles/hill were very low and % filled grains was only 73% resulting in a very low grain yield of only 3.54 t ha^{-1}. An unbalanced distribution of assimilates resulted in higher biological yield than economic yield with a harvest index of 0.45.

Dry Matter Yield and NPK Concentration and Uptake in Rice

Dry matter yield, NPK concentration and uptake in rice grown after vetiver during 2018 DS are presented in Table 7 and Figure 4. As expected, root biomass of NPK concentration and uptake were lowest in roots. Straw dry matter yield was 3396 kg ha^{-1} while grain yield was 3540 kg ha^{-1}. Higher N and P concentration and consequently uptake was recorded in grain with 45.67 kg N ha^{-1} and 15.22 kg P ha^{-1} compared to 26.49 kg N ha^{-1} and 7.47 kg P ha^{-1} in straw. On the other hand, K concentration (1.28%) and uptake (43.47 kg ha^{-1}) was found in rice straw. The lower translocation of K to the grain resulted in a lower grain yield as shown in Table 6. K is very important in dry matter production and is involved in active translocation of assimilates during grain filling. This also contributed to lighter weight of 1000 seeds, which are less filled and with smaller grain size (Table 6). Results indicate that the nutrients naturally found in the soil were fully utilized by vetiver for their higher biomass since vetiver was not fertilized.

Table 6: Agronomic parameters and yield components of rice grown after vetiver during 2018 DS (vetiver-rice)

PLANT HEIGHT (cm)			NUMBER OF TILLERS	PANICLE COUNT (no./hill)	SPIKELET (no./ panicle)	FILLED GRAINS (%)	1000 SEED WT.(g)	BIOMASS (kg ha^{-1})	GRAIN YIELD (t ha^{-1})	Harvest Index			
Vegetative (14DAT)	Booting (50DAT)	Harvest (87DAT)	Vegetative (14DAT)	Booting (50DAT)									
34.02	80.15	98.6	5.2	14.2	13.2	102	72.07	22.85	624.01	849	3396	3.54	0.45

Table 7: Dry matter yield, NPK concentration and uptake distribution of nutrient in root, straw and grain of rice grown after vetiver during 2018 DS.

GROWTH STAGE	PLANT PART	DRY MATTER (kg ha^{-1})	CONCENTRATION (%)			UPTAKE (kg ha^{-1})		
			N	P	K	N	P	K
Maturity (58-88DAT)	Straw	3396	0.78	0.22	1.28	26.49	7.47	43.47
	Root	849	0.63	0.19	0.29	5.35	1.61	2.46
	Grain	3540	1.29	0.43	0.23	45.67	15.22	8.14

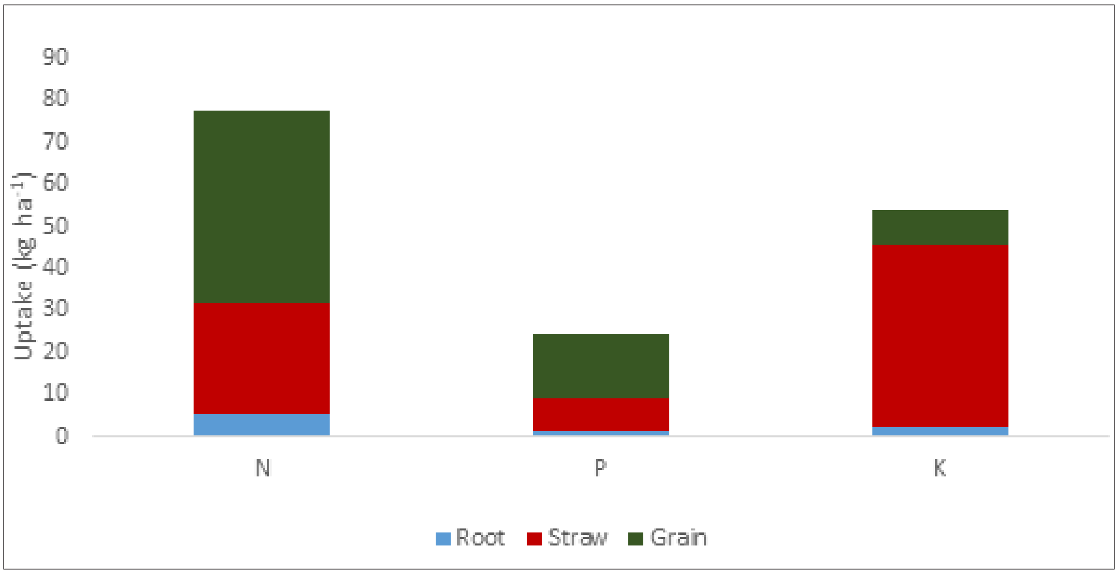

Figure 4: Nutrient partitioning in rice grown after vetiver during 2018 DS.

Distribution of Cr and Ni in Rice Grown after Vetiver (2018 DS)

Cr and Ni concentration and uptake in rice grown after vetiver during 2018 DS at various growth stages is shown in Table 8 and Figure 5. During the vegetative stage, roots had the highest Cr concentration (456.5 mg kg^{-1}) and uptake (0.05 kg ha^{-1}) compared to straw with a concentration of 21.5 mg kg^{-1} and an uptake of 0.01 kg ha^{-1}. At panicle initiation, more Cr still concentrated in the roots (322 mg kg^{-1}), resulting to an uptake of 0.25 kg ha^{-1}. At maturity, Cr was only detected in the roots and straw and was not translocated to the grain. A similar trend was observed for Ni. At vegetative stage, roots had the highest Ni concentration (339.5 mg kg^{-1}) and uptake (0.04 kg ha^{-1}) compared to straw with a concentration of 15 mg kg^{-1} and an uptake of 0.01 kg ha^{-1}. During panicle initiation, more Ni still concentrated in the roots (359.5 mg kg^{-1}) resulting to an uptake of 0.28 kg ha^{-1}. At harvest, Ni was only detected in the roots and straw and was not translocated to the grain. Results imply that at the vegetative stage, the rice plant was so active in absorbing nutrients including these heavy metals. Through time and as the growth of plant progresses, nutrient uptake also increases, particularly at reproductive stage wherein uptake is higher for growth stabilization. The grains were below detection limit in both Cr and Ni because these metals were immobilized by the roots. Some of the Cr and Ni could have been present in the rice bran and in unfilled grains.

Table 8: Distribution of Cr and Ni in root, straw and grain of rice grown after vetiver during 2018 DS at various growth stages.

GROWTH STAGE	PLANT PART	DRY MATTER(kg ha^{-1})	CONCENTRATION (mg kg^{-1})		UPTAKE (mg ha^{-1})	
			Cr	Ni	Cr	Ni
Vegetative (14 DAT)	Straw	515.68	21.5	15	0.01	0.01
	Root	112.32	456.5	339.5	0.05	0.04
Panicle initiation (50DAT)	Straw	3160.3	5	5	0.02	0.02
	Root	789.64	322	359.5	0.25	0.28
Maturity (58-88DAT)	Straw	3396	2.5	2.5	0.01	0.01
	Root	849	216.5	179	0.18	0.15
	Grain	3540	BDL	BDL	BDL	BDL

BDL: Below Detection Limit.

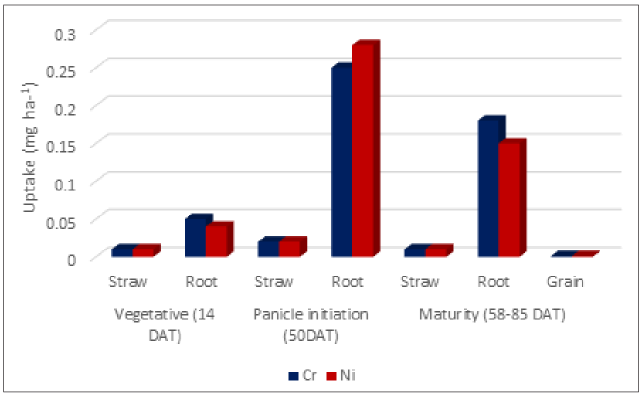

Figure 5: Heavy metal partitioning in rice grown after vetiver during 2018 DS (vetiver-rice) at various growth stages.

Bioaccumulation and Translocation Factors of Rice after Vetiver (2018 DS)

The bio-accumulation and translocation factors of rice grown after vetiver are shown in Table 9. Bio-accumulation factor has been calculated for the transfer of heavy metals from the soils to the different parts of rice. For Cr, BAF was only 0.14 and 0.18 for Ni indicating that rice absorbs Cr but does not store it. When BAF is <1 or =1, it indicates that the plant only absorbs but does not store heavy metals. However, if BAF is >1, it is an indication that the plant stores metals. The transfer of metals from the soil to plant is one of the key elements of human exposure to metals via the food chain (Dixit et al., 2001).

Translocation factors for both Cr and Ni were below the detection limit (BDL), indicating that these metals were not translocated to the edible portion of the plant, particularly the rice grain. The translocation factor reflects the transfer of metals from the roots to the edible parts, wherein different plants display different properties with regard to its root activities and exudates. The roots contribute greater impact on the solubility and phyto-availability of metals in the soil, thus Cr and Ni levels were below the detection limit in the grain.

Table 9: Bio-accumulation and translocation factors of rice grown after vetiver during 2018 DS.

FACTOR	CHROMIUM	NICKEL
Bio-accumulation Factor	0.14	0.18
Translocation Factor	BDL	BDL

BDL: Below Detection Limit.

Effect of Vetiver-Rice Rotation on Soil Properties

Table 10 shows the soil parameters as affected by growing rice after vetiver during 2018 DS. Soil pH increased to 6.53 and organic matter content improved significantly (48% increase) which is due to the unique characteristics of the vetiver roots. Total Cr was reduced by 353 mg kg^{-1} (8.18 %) and available Cr by 282 mg kg^{-1} (15.04%) while total Ni declined by 742 mg kg^{-1} (28.02) and available Ni by 723 mg kg^{-1} (41.91 %). Results implied that vetiver plant grown for a short duration (3 months) has the capability to phytoremediate Cr and Ni-contaminated soil. This is one of the most cost-effective green technologies and most useful method for decontaminating the soil, wherein Cr (VI) are converted into non-toxic Cr (III) according to Chaney et al. 2007; Bolan et al. 2003b; Dalton et al. (1996) reported that vetiver grass that has high root biomass can accumulate a significant amount of Cr. This plant tends to reduce metal toxicity by converting highly toxic and readily mobile metals into less toxic ones.

Table 10: Soil parameters as affected by rice grown after vetiver during 2018 DS (vetiver-rice).

SOIL PARAMETERS	INITIAL	AFTER VETIVER	INCREASE/ DECREASE (3 MONTHS) AFTER VETIVER	RICE AFTER VETIVER 2018 DS	INCREASE/ DECREASE AFTER 2018 DS RICE	TOTAL INCREASE/ DECREASE AFTER ETIVER AND RICE
Soil pH (H_2O)	5.1	6.21	1.11	6.53	0.32	1.43
Soil organic matter (%)	1.76	2.6	0.84	2.7	0.1	0.94
Total Cr (mg kg^{-1})	4314	4128	-186	3961	-167	-353
Total Ni (mg kg^{-1})	2648	2387	-261	1906	-481	-742
Available Cr (mg kg^{-1})	1875	1715	-160	1593	-122	-282
Available Ni (mg kg^{-1})	1725	1479	-2460	1002	-477	-723

Economic Analysis

Return on investment describes the ratio of net income with total expenses. In economic view, this means that as much as Php 0.50 can be gained for every peso investment when planting rice after vetiver for one (1) cropping season. The vetiver plant did not receive fertilizer or any soil amendments. However, after harvesting vetiver, rice was planted and fertilized using rice crop manager (RCM) as shown in Table 11.

Table 11: Economic analysis of rice production as affected by phytoremediation management in Lomboy Sta. Cruz, Zambales.

	GRAIN YIELD	TOTAL EXPENSES	FERTILIZER APPLIED	GROSS INCOME*	NET INCOME	ROI**
	kg ha^{-1}	(P ha^{-1})	(P ha^{-1})	(P ha^{-1})	(P ha^{-1})	
Vetiver-rice	3540	42 462.5	11 762.25	63720	21257.5	0.5

*Computed based on the prevailing price of palay in the area @18.00 kg^{-1} **ROI-Return on Investment ha^{-1}

Conclusion

Based on the results of the study, the rice field was highly contaminated with Cr and Ni due to its naturally occurring mineral from the parent material and the presence of mining activities. Remediation technique like phytoremediation with the use of vetiver was ideal and sustainable alternative on the permanent removal or recovery to rice paddies. These were significantly increased soil pH and percent organic matter with decreased amount of total and available Cr and Ni.

The use of vetiver implied on the bio-accumulation of Cr and Ni that this plant has the capacity to absorb and store metals on their system particularly the roots. With the continuous cultivation of vetiver in the field higher bio-accumulated metals were observed due to its increasing biomass. However, the translocation factor in both seasons (WS and DS) show that it is below 0.02 mg kg^{-1} as prescribed limits fixed by the Codex Standard and European Union (EU Standards). Vetiver plant had a lower translocated Cr and Ni to their shoots thus it is safe for forage purposes. It was observed that the longer the time of vetiver in the field the lower is the translocation factor due to that fact that vetiver has high capacity to store and uptake metals according to its bio-availability.

The use of rice after one cropping of vetiver (vetiver-rice) in mitigating Cr and Ni indicate that the plant only absorbs (Cr and Ni) particularly more on the roots. The translocated amount was reduced to grain because the roots contribute greater impact on the solubility and phyto-availability of metals in the soil thus Cr and Ni were below detection limit on the grain.

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