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Phytoremediation potential of C*entella asiatica* (gotu kola) in nickel ore-contaminated soils

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Key words: Background soils, *Centellaasiatica*, Nickel ore, Contamination factors.

Abstract

Nickel miningposed a serious environmental problem due to run-offs and tailings. To address this, current techniques include excavation, chemical stabilization and soil flushing, but these methods are costly and impractical. One of the ecologically accepted treatments is phytoremediation. With the capacity of *Centella asiatica* (gotu kola) to thrive in moist soils with domestic effluents, this present study sought to evaluate its phytoremediation potential by employing an experimental design with three replicates of: (a) nickel-rich bio-ore soils from the mining site in Carrascal, Surigao del Sur as treatment substrates; and (b) natural background soils from Iligan City as the control substrate). Phytoremediation potential of *C. asiatica* was assessed through relative plant growth, bioaccumulation capacity through Atomic Absorption Spectrometer (AAS), contamination factor (CF) computationand tolerance-accumulating mechanism through SHAPE software tool which evaluates shape variations based on elliptic Fourier descriptors. Results reveal relative growth values close to 1 which means that they have the potential to survive in nickel-contaminated condition. AAS results show a greater decrease in soil nickel content and a bigger increase in nickel accumulation in the plant samples in the nickel-ore contaminated soils than in the background (control soils). Contamination factor values indicate that soil and plant samples have very high contamination factor ($6 < CF$). SHAPE analysis between the control and treatment set-up shows no variations ($p= 0.155$) in the leaf shape of *C. asiatica* which indicates its tolerance-accumulating mechanism. These concerted results suggest that *C. asiatica* may exhibit phytoremediation potential in nickel-ore contaminated soils.

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Introduction

Nickel contamination is a very important environmental problem though, the fact remains that it is extremely difficult to remediate the heavy-metal contaminated soils. Current techniques used to remediate heavy-metal contaminated soils include excavation, chemical stabilization, soil washing or soil flushing, but these methods are costly and impractical (Mehes-Smith *et al.,* 2013).

Phytoremediation is an ecologically acceptable process and cost-effective use of hyper-accumulator plants to remediate contaminated soils (Lone *et al.,* 2008; Sharma *et al.*, 2013).

Centella asiatica (gotu kola) is a small, herbaceous plant usually seen in shady and moist areas and can even thrive abundantly in canals with domestic effluence. In the context of phytoremediation, it was reported to be accumulate copper, lead, and zinc in contaminated media (Yap *et al.,* 2010; Mokhtar *et al.,* 2011a,b;Bahnika&Baruah 2014;).

This however, is still a less studied plant in the field of phytoremediation, especially in the case of nickel.

Carrascal nickel mining sites in Surigaodel Sur, Mindanao, Philippines is one of the worlds' largest producers of nickel (US Geological Survey, 2015).

Current mining operations are all conducted above ground however; reported nickel mobility includes erosion and run-offs through river systems, estuaries and finally oceans, the ultimate sink.

It can also enter groundwater supplies by leaching through the soil column. Soils in agricultural areas are becoming inappropriate for sustainable agriculture due to siltation which leads to phytotoxicity (NSCEP-EPA, 1990). With this present condition, this study sought to evaluate the possibility of using *Centella asiatica* (gotukola) for phyto-remediating nickel-rich bio-ore contaminated soils though an experimental design.

Materials and methods

Study sites

The test site selected for the study is the Carrascal Nickel Mining Site in Surigao del Sur, Philippines with Global Positioning Coordinates (GPS) readings N 09° 35656', E 125° 91590 (Fig. 1).

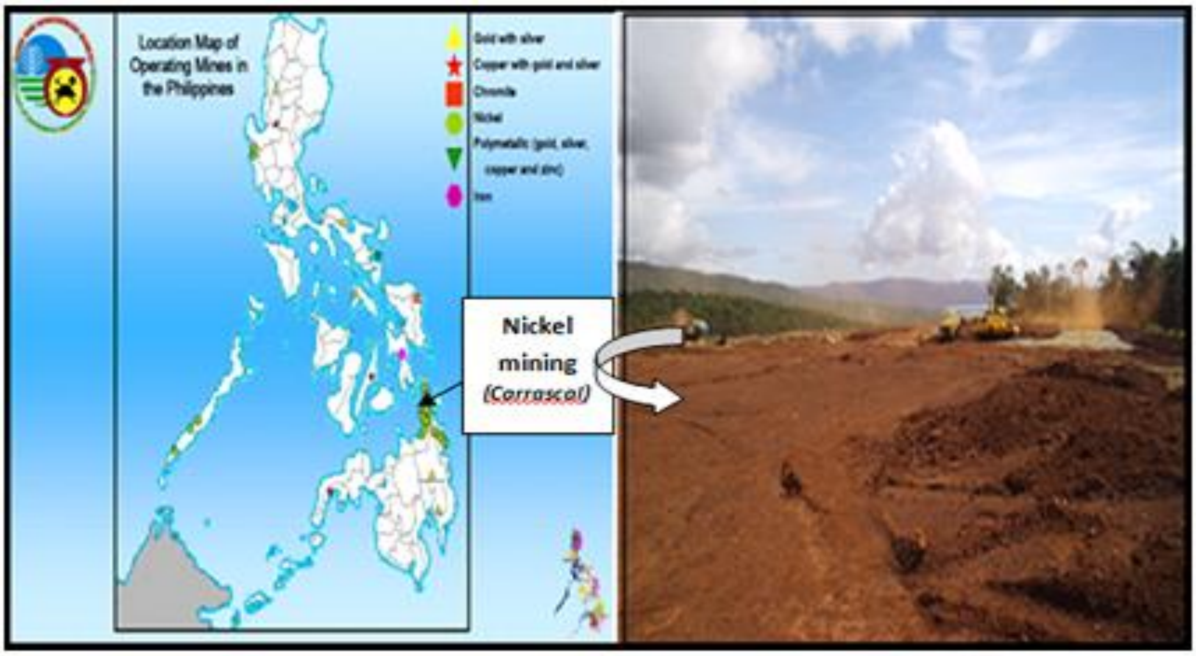

Fig. 1. Map showing the operating nickel mining sites in Carrascal, Surigao del Sur, Philippines.

This site was selected for the treatment set-up because of its high soil nickel content.The background (control) site selected for the study is the natural dwelling soils of *C. asiatica* in Iligan City, Philippines which is distant from any anthropogenic source of metal contamination.

Collection of soil samples

Nickel-rich bio-ore soils were collected through scoop method from the mining site in Carrascal, Surigaodel Sur and from the natural dwelling soils of *C. asiatica* in Iligan City. The soil samples were air- dried at room temperature for two weeks. Fig. 2a-b shows the soil samples.

Carrascal soil exhibits distinct reddish color owing to its metalliferous type (Fig. 2a) while the natural dwelling soil of *C. asiatica* is muddish black in color (Fig. 2b).

At each location, eight random partial soil samples weighing 0.5 kg each were collected from 0 to 20 cm depth and were mixed to obtain one composite sample to save time and costs. One hundred (100) grams of each soil type were air-dried and placed in polyethylene bags for initial nickel content analysis.

Plant material

C. asiatica were uprooted from its natural dwelling habitats within 5-15 cm depth rooting zone. These plants were put in a hydroponic system containing tap water for a two-week acclimatization period before exposing to heavy metal contaminants. Fig. 3a-b shows the plant habit of *C. asiatica* in its actual dwelling soils and the process of acclimatization.

Approximately 250-300 grams of the plant were put in each container and allowed to grow, being watered with two hundred fifty (250) ml of water daily for 21 days. The experimental design was patterned after the works of Mokhtar *et al.* (2011a,b) where three (3) replicates were employed in each treatment set-up (nickel-ore contaminated soils) and control (background soils).

Relative plant growth assessment

Relative growth assessment refers to the comparison of the various increases in weight of *C. asiatica* at different time interval. This method is necessary to determine if the test plant has the potential to survive in a nickel-contaminated soil and to evaluate initial nickel toxicity. This was done by monitoring the wet weight of the plants at the start and at the end of the experimental set-up and was calculated as Wf / Wi where:

Wf =is the final wet weight of plants after exposure to contaminant;
Wi =is the initial weight of the plants.

Relative growth values close to 1 indicates that the used test plant has the potential to survive in the contaminated condition aside from accumulating high levels of the toxic contaminant.

Plant and soil samples pre-treatment

After 21 days of exposure to contaminants, *C. asiatica* were harvested and washed with running tap water to remove adhered soils and then washed with distilled water. The plant samples (at least 80g of the air-dried plant specimen) were placed in polyethylene bags for storage until later analysis.

Bioaccumulation capacity assessment by Atomic Absorption Spectrometer (AAS)

Heavy metal concentration (nickel) in soil and plant samples was determined through Atomic Absorption Spectrometer (AAS). Air-dried plant samples were further dried in an oven at 65°C for 24 h. It was then grounded and was put in a flask (about 1-5g). The sample must undergo acid extraction as patterned after the "Official Method of Analysis of AOAC International (19th ed., 2012). In this study, a mixture of 10 ml concentrated H_2SO_4 and 5 ml of concentrated HNO_3 were added to have it digested. This solution was then heated in an oven at low temperature for about 14 hours until the sample dissolved completely. Approximately 10ml of 30% H_2O_2 was added and allowed to cool. On the other hand, 100g of the soil samples were dried in an oven for 6h at 105°C. The dried soil samples were crushed and analyzed for Ni concentration.

Quantification of heavy metals was based upon calibration curves of standard solutions of respective heavy metals. These calibration curves were determined several times during the heavy metal analysis and controlled by including triplicate samples in analytical batches and blanks.

Evaluation of Contamination Factor (CF)

The Contamination Factor (CF) has been used to assess soil contamination (Agunbiade & Fawale 2009; Chandran *et al.*, 2012; Aartri *et al.*, 2012; Das & Shil2012) through comparison of the concentrations in the surface layer to background values (control) by the expression:

$C^i_f = \underline{C^i_{0-1}}\, C_n^i$

Where, C^i_f is the contamination factor (CF), C^i_{0-1} is the mean of the concentrations of individual metal from all test sites and $C_n^i$is the baseline or background concentration of the individual metal. This expression was also adapted to calculate CF in plant samples. CF was defined according to four categories as follows:

CF < 1 - Low contamination factor

1 < CF < 3- Moderate contamination factor

3< CF< 6- Considerable contamination factor

6< CF - Very high contamination factor

Shape analysis

Image acquisition was done in each of the 30 leaf samples from the two set-ups (nickel-ore contaminated soils and background soils). The outline of the leaf samples were analyzed in chain coding technique using the software package SHAPE v.1.3 (Iwata & Ukai, 2002) to examine the tolerance-accumulating mechanismin terms of its shape variation. All images were saved in .bmp format (24bit) and were binarized with Chain Coder. Chain code is a coding system for describing geometrical information about contours in numbers from 0 to 7. Chain coder converts the full color image into a binary (black and white) image, reduces noise, traces the contours of objects and describes the contour information as chaincode. Then, the Chain-code file was transformed into a Normalized Elliptic Fourier file with Chc2Nef, using 20 harmonics. These Elliptic Fourier descriptors (EFDs), originally proposed by Kuhl & Giardina (1982), can delineate any type of shape with a closed two-dimensional contour.

Normalization of data obtained from chain codes used the first harmonic ellipse as a basis which corresponds to the first Fourier approximation and utilized the 20 harmonics number to be calculated as suggested by Iwata and Ukai(2002). It is based on the methodology of Elliptic Fourier descriptors which allows describing each type of two-dimensional shape with a closed outline, in terms of harmonics (Joaquino *et al.*, 2017). It allows detailed analysis of fine-scale morphological variation in the outline of the dorsal shell part of the giant African snail. The matrix of the harmonic coefficients underwent normalization based on the first harmonic, the data transformed into shape variables. Subsequently, a Principal Component Analysis (PCA) was performed on the variance-covariance matrix of normalized coefficients (elliptic Fourier descriptors) using Prin Comp, which gives a graphical output of the average shape the standard deviation (Magrini & Scoppola, 2010. Principal component scores were further subjected to Multi-variate Analysis of Variance and Canonical Variate Analysis (MANOVA/CVA) using PAST ver. 1.91 as platform (Hammer *et al.*, 2001) to determine if the populations differ significantly from one another based on the shape of its shell. Wilks' lambda, Pillai trace values and p values were also obtained.

Results and discussion

Relative plant growth

Relative plant growth is one of the parameters evaluated if it can survive in a heavy-metal contaminated condition. *C. asiatica* demonstrates one of the initial and important requirements in phytoremediation process - the capacity to survive in a contaminated condition (Fig. 4).

Figure 4reflects the healthy leaves of *C. asiatica* with new shoots sprouting in 7th day (a), 14th day (b) and even during harvesting period on the 21st day (c).Table 1 shows the initial and final wet weight of *C. asiatica* after exposure to nickel contaminant for 21days, as well as its computed relative growth.

Table 1. Wet Weight of *Centella asiatica* before and after exposure to nickel contaminant and its computed relative growth.

Treatment	Initial weight (g)	Final weight (g)	Relative growth
C. asiatica (Nickel-ore contaminated soils)			
R1	252.5	328.51	1.30
R2	292.82	431.49	1.47
R3	255.52	292.71	1.15
*C. asiatica (*background soils)			
R1	255.4	310.2	1.21
R2	276.7	345.5	1.25
R3	298.01	380.64	1.3

R – replicates.

Table 2. Initial and final nickel concentration in soil samples after treatment with *C. asiatica* plants.

C. asiatica set-up	Initial Nickel Soil Content	Final Nickel Soil Content	Computed Difference
Nickel-ore contaminated soils	5, 457.0 mg/kg	4, 659.0 mg/kg	798 mg/kg
Background soils (Control)	103.5mg/kg	94.4mg/kg	9.1 mg/kg

Since the relative growth of all plants were close to 1, the test plant shows potential to be used in the phytoremediation system which requires plants to be able to accumulate acceptable amount of metals and also to survive in the contaminated condition. Table 2 shows the initial and final nickel concentration in soil samples after the experimental set-up. Initial nickel content of nickel-ore contaminated soils is 5, 457.0 mg/kg, which is almost fifty-three times greater than the natural dwelling soils of *C. asiatica* from background (103.5 mg/kg).

Metalliferous, serpentine soil usually contains 400-6,000 mg/kg nickel while natural levels of nickel in loam and clays ranged from 90-100 ppm.

Table 3. Nickel content in *C. asiatica* after treatment.

C. asiatica set-up	Nickel Content in *C. asiatica* (in mg/kg)			Mean
	R1	R2	R3	
Nickel-ore contaminated soils	65. 90	179.70	432.00	225.87
Background soils (control)	7.60	11.50	9.40	9.50

R – replicates

Table 4. Contamination Factor (CF) in plant and soil samples.

Samples	Contamination Factor (CF)
Soil samples	52.72
Plant Samples (*C. asiatica*)	23.78

This indicated that elevated nickel is present in the treatment soils while the background soils were less stressed by environmental contaminations and was suitable for use as a control or reference site (Aurangzeb *et al.,* 2014). Reduction in nickel soil content after treatment with *C. asiatica* is also an indication if phytoremediation is evident (Quian *et al.*, 1999; Garbisu and Alkorta, 2001; Ghosh and Singh, 2005; Mokhtar *et al.*, 2011a,b;).

Table 5. MANOVA results between the two populations of *C. asiatica* based on significant relative warp (RW).

Wilks lambda	df1	df2	F	p(same) α – 0.05
0.7326	11	46	1.527	0.155
Pillai trace				
0.2674				

All treatments showed decrease in soil nickel content however a substantial reduction of 798 mg/kg is evident in the treatment soils while a slight decrease was obtained in the control soils (9.1 mg/kg). This decrease in soil nickel content is an evidence of the bioaccumulation activity of *C. asiatica* when introduced in nickel-ore contaminated soils. These results agree with the works of Yap *et al.* (2010). Table 3 shows the bioaccumulation values of the plant samples after 21-day treatment.

Fig. 2. Nickel-rich soil samples (a) and soil from the natural dwelling habitat of *C. asiatica* (b).

As shown, the range of nickel accumulation in the plant samples presented was generally higher in the treatment soils than in the background (control soils). The highest concentration of Ni was reflected in the *C. asiatica* harvested from nickel-ore contaminated soils (432.0 mg/kg). This higher nickel removal at greater concentration of Ni in soils was due to the loading effect where the sorption sites were saturated by nickel at the highest concentration (Mokhtar *et al.*, 2011a, b).

his is also supported by Robinson (1997) who states that the metal concentration in plants is proportional to the extractability of the metal in the soil.

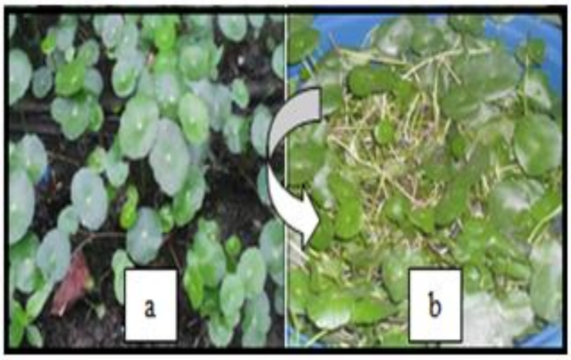

Fig. 3. Plant habit (a); acclimatization of *C. asiatica* (b).

Brown *et al.* (1987) states that typical nickel toxicities occur in woody plants if tissue levels exceed 80-120 ppm. Sensitive plants, such as tomato, may exhibit toxicities above 10 ppm in their tissues. Hence, these obtained nickel levels in *C. asiatica* treated with nickel-ore contaminated soils is beyond the tolerable amounts. However, it could be that they develop certain physiological mechanism to be able to resist nickel toxicity. According to Mehis-Smith *et al.* (2013), heavy metal resistance can be achieved by avoidance and/or tolerance. Avoidance mechanism prevents metal ions from entering their cellular cytoplasm, while tolerance mechanism detoxifies metal ions that have crossed the plasma membrane or internal organelle bio membranes. Further, according to Wei *et al.* (2005), the strategies used by plants in metalliferous soils fall into three categories: metal excluder, indicators and accumulators/hyper accumulators. With these different categories specified, *C. asiatica* could be using tolerance-accumulating physiological strategy by accumulating nickel in their harvestable biomass.

Fig. 4. Growth of *C. asiatica*at 7 days (left), 14 days(middle) and 21 days (right).

Contamination Factor (CF)

The contamination factors (CF) were calculated for both the plant and the soil samples (Table 4). Contamination Factors (CF) data supports that soil and plant samples have very high contamination factor (6 < CF).

Favas *et al.* (2014) states that vegetation covers such as this herbaceous *C. asiatica* can be established on highly contaminated landfills and tailings.

Shape analysis

Figure5 shows the contour shapes of the leaf samples of *C. asiatica* after treatments.

There are ten (10) significant principal components (PCs) obtained using the software SHAPE v.1.3 by Iwata & Ukai (2002).In the determination of subtle variations between the two populations, these PCs that defined shape differences were used.

This became an exploratory procedure in order to create comparison between shapes and elucidate possible biological significance. Tables 2 & 3 show the MANOVA results between the four populations of *A. fulica*.

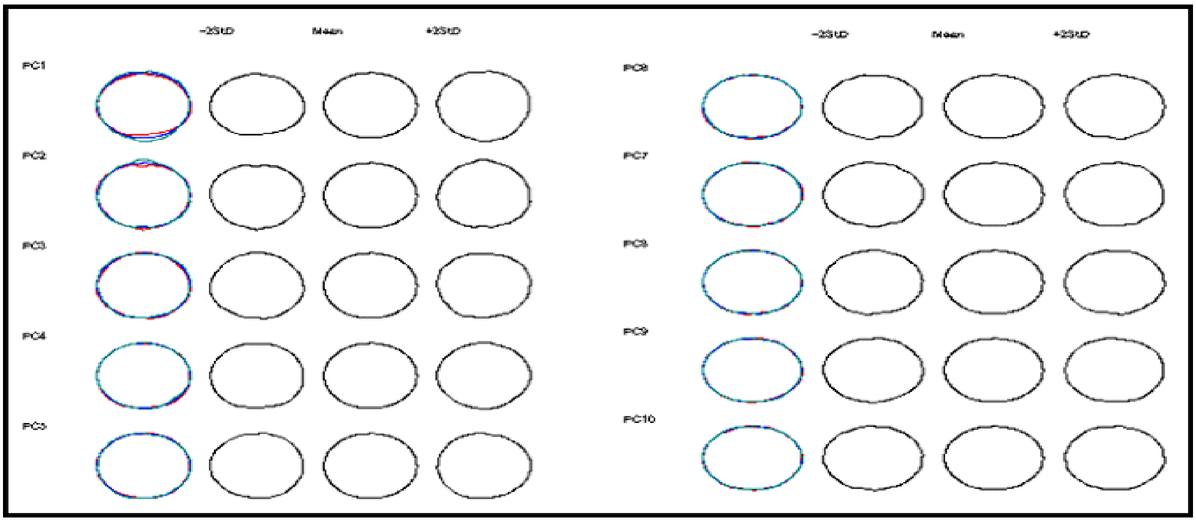

Fig. 5. Contour shapes of the leaf samples of *C. asiatica.*

The obtained value ($p = 0.155$ at $\alpha - 0.05$) indicates no significant difference in terms of the *C. asiatica*leaf sample shapes after being planted in two conditions (nickel ore contaminated soils and background soils).

This leaf shape analysis using a computer software package (SHAPE v. 1.3) indicates the capacity of *C. asiatica* to tolerate varying soil conditions.

Conclusion

C. asiatica exhibits high levels of nickel tolerance since it is able to survive in a nickel-contaminated soil. A decrease in the soil content and bioaccumulation of nickel in this test plant beyond the tolerable amount after treatment also supports its phytoremediation potential. Further, its capacity to tolerate varying soil conditions was evident through shape analysis. Hence, *C. asiatica* could be a phytoremediation tool to detoxify nickel-contaminated soil.

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