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PHYTOREMEDIATION POTENTIAL OF SEAGRASSES AND SEAWEED SPECIES IN THE COASTAL RESOURCES OF BRGY. BOLITOC, STA. CRUZ, ZAMBALES, PHILIPPINES

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ABSTRACT: Phytoremediation is a new technology that opts to contain, destruct and or extract pollutants from the environment thru photosynthesizing organisms. Sea grasses and seaweeds, as marine organisms, are adaptive organisms with their respective ecological niches, holding an important role in the whole biodiversity of the ocean. In this paper, the goal was to assess the capability to absorb nickel and potentiality as phytoremediator of the two types of marine organisms, sea grass and seaweed. Three sea grasses and four seaweeds in the area near the vicinity of nickel stockpile in Sta. Cruz, Zambales were collected, prepared and analyzed for their capability to accumulate nickel. The analysis was thru the use of Flame Atomic Absorption Spectrophotometer which quantified the amount of nickel in each of the species' dry matter. Results of the study revealed that there was nickel present in whole plants of each sea grass and seaweed species. It showed that the three sea grassses (*Cymodocea rotundata*, *Thalassia hemprichii* and *Halodule pinifolia*) and four seaweeds (*Sargassum ilicifolium*, *Sargassum fulvellum*, *Sargassum olicystum* and *Padina australis*) were able to contain nickel in their tissues. For the sea grasses, *H. pinifolia* showed the capability to absorb nickel higher than the threshold value while same is true for the four seaweeds, *S. ilicifolium*, *S. fulvellum*, *S. olicystum* and *P. australis*. Hence, the sea grasses and seaweeds found in the area have the capability to absorb nickel from the coastal areas for water and sediment conservation.

Key words: phytoremediation, sea grass, seaweed, nickel

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INTRODUCTION

Marine contamination has been one of the persistent and serious problems of our environment. Solid and liquid wastes, from industrial, agricultural and residential areas, greatly affect our ocean, making it the sink for all contaminants. Environmental contamination is composed of different pollutants such as, organics, salts, heavy metals, trace elements and radioactive compounds in varying quantities. Heavy metals is a prevalent group of pollutants which are generally found underneath the earth, usually find way to the surface due to the malpractice of waste disposal, domestic and agricultural use, industrial production and unguided mining operations. Few metals are toxic and lethal in trace concentrations and these may cause teratogenic, mutagenic, endocrine disruptors while others can cause behavioral and neurological disorders among infants and children [17].

Moreover, unlike organic compounds, heavy metals are non-biodegradable; therefore the removal of these pollutants includes a rigorous process to be removed from the environment. Unfortunately, present solution for a cocktail of contaminant such as heavy metals in the environment is thru chemical and thermal methods that are technically difficult, time-consuming, environmentally difficult and expensive [9,12]. However, existing remediation strategies costs a lot. This results to the search for environmental clean-up strategies that are cheap but are highly effective in terms of environmental restoration.

Phytoremediation is a novel, eco-friendly new way of cleaning-up the environment. It is known for its cost-effectiveness, aesthetic effect and good public acceptance for the reason that it's solar-driven biotechnological application that uses plants and associated soil microbes for detoxification, removal or immobilization of organic, nutrient and metal pollutants [25,1,12]. Due to wide industrialization of the whole world, pollutants are piling up on earth's surface resulting for the need of technologies that will step-by-step address chemical fertilizers, pesticides, industrial waste materials and sewages, organic and inorganic pollutants. Discovery of hyperaccumulators plants widely open the doors for opportunity for phytoremediation and phytomining which are both very much beneficial for us and the environment when used as a solution to our contamination problems [1]. Phytoremediation, on the other hand, has been understood as a technology that makes use of plants; hence, it is sometimes also called as "green technology". In this technology, photosynthetic organisms absorb contaminants from the site or media [20] and effectively sequester these from the environment leaving only healthier and more human-friendly surrounding. Many studies on plants for phytoremediation are on record outside the Philippines, but quite few were found locally.

Fig. 1 Map of Zambales and the Philippines

Philippines, an archipelagic country, has a wide marine area that serves as one of its food resources. Being a very much developing country [11], Philippines is also struggling in terms of mitigating the pollution it is currently facing, therefore, a need for a low-cost, efficient technology that has the capacity to start and eventually resolve environmental degradation.

As a mining location, Sta. Cruz, Zambales experiences adverse effects from the improper handling of unearth nickel ore that are transferred from the mountain to the stockpile which, on the other hand is near the shore of Brgy. Bolitoc, Sta. Cruz, Zamables and the residential community. During the transfer, dusts from the mined material are being dropped off to places included in the route of trucks used by the mining companies. Due to this situation, nickel dusts eventually piled-up and became contaminants of Sta. Cruz, Zambales.

This study attempted to verify nickel contamination in the coastal area near the stockpile in Brgy, Bolitoc, Sta. Cruz, Zambales, quantify nickel content in the tissues of sea grasses and seaweeds that thrive along the study location and prove the potentiality of these marine organisms as phytoremediation agents for nickel allegedly deposited to the site by its neighboring nickel stockpile.

MATERIALS AND METHODS

Species Survey

Sta. Cruz (15°45'59.20"N 119°54'59.98"E) is the last municipality of Zambales situated at its northern end which has 25 barangays (Figure 1). It is a coastal municipality that supports fishing, agriculture, tourism and mining as the livelihoods of its people. The study took place in September 2015 in the coastal area of Brgy. Bolitoc, Sta. Cruz, Zambales (15°44'55.36"N 119°53'12.01"E), where beds of sea grasses and seaweeds are present near the stockpile (Figure 1).

Sea grasses and seaweeds that were present in the coastal area were surveyed using two different methods. Sea grass survey was done using a transect line of 100 meters and quadrat of 0.25 m^2 which has 25 subquadrats [4]. Three transect lines were laid out with a distance of 150 meters from each other. Four quadrats were positioned along the transect lines, with equal distance in between each quadrat. For seaweeds, a quadrat of 1m x 1m was laid out ten times for the seaweed species survey in the area of study. Species of sea grasses and seaweeds present in the quadrats during their respective surveys were noted. Both types of species were pre-identified thru their morphological characteristics using manuals i.e. Field Guide & Atlas of the Seaweed Resources of the Philippines [24] and Sea grasses of the Great Barrier Reef [15]. The identification and classification of all species were verified by an expert at the National Museum, Manila.

Sediment and Water

The water and sediment samples of both ecosystems were sampled for the confirmation of nickel contamination in the area. Water samples were collected thru bottle submersin method, a protocol adopted from CRL Laboratory, Clarkfield, Pampanga. The technique was done by submerging the bottle 0.10m below the water surface [8]. Approximately 100 mL of water sample were collected from each of the quadrats used in each ecosystem. The composite samples of water were labeled with the site of collection: seagrass ecosystem and seaweed ecosystem. On the other hand, sediment samples were collected using large spoon method which was also adopted from the CRL Laboratory, Clarkfield, Pampanga. Sediment from each quadrat throughout the study were sampled. The samples from the seagrass ecosystem and seaweed ecosystems were combined, respectively, and these were labeled with the site of collection.

The water and sediment samples were temporarily stored in an ice chest to preserve freshness until the transport to CRL Laboratory, Clarkfield, Pampanga for the analysis. Nickel analysis for each sample was done through the use of Flame Atomic Absorption Spectrophotometer (FAAS) equipment model Shimadzu A7000.

Sea grass and Seaweed Sampling and Analysis

Sea grass and seaweed species present in the study site were subjected for analysis to determine which among these could accumulate nickel and have the potential to phytoremediate nickel present in the site. Sea grasses and seaweeds were collected using sharp knife. Samples for each species were collected and placed in net bags.

Collected sea grass samples were thoroughly washed with tap water and a phosphate-free soap to get rid of the sediments trapped on the surface while they were submerged underwater. Seaweed species were also washed to get rid off of trapped dirt, however, these were not cleansed with soap to avoid soap diffusion inside the specimen's tissues. After this, all samples were rinsed with distilled water [13] and were drained off as preparation for the drying method. After washing, the samples were allowed to be air-dried to let more excess water to evaporate. Next, these were cut into smaller pieces for faster drying prior to oven-drying at 60^0 C [13] until samples were crisp dry. Afterwards, dried sea grass and seaweed samples were pulverized using a heavy duty blender. The samples were prepared at the Biodiversity Molecular Laboratory, Biodiversity Center, Institute for Climate Change and Environmental Management, Central Luzon State University.

Following the preparation, the samples were delivered to CRL Laboratory, Clarkfield, Pampanga for analysis. Nickel content of each seaweed species sample was analyzed using Shimadzu A7000 FAAS.

RESULTS AND DISCUSSION

The contamination of Ni in the coastal areas near the stockpile in Brgy. Bolitoc was confirmed thru the analysis of both water and sediment samples from each area. Due to the study sites' proximity to the stockpiles of Ni ore, residues tend to accumulate to the surrounding water and sediment; this occurrence eventually results to problems that may concern the people, the environment as well as the organisms that inhabit the area.

Sea grass

Ni contamination in sea grass area

Both sediment and water samples from the sea grass ecosystem came out positive with nickel. Figure 2 shows that the concentration of nickel in water was only 1.1ppm which, however, exceeded the threshold limit of 0.02ppm for water medium. Moreover, it is also shown that the sediment has a quantified amount of nickel at 238ppm that also exceeded its limit of 110ppm. In this regard, it is confirmed that the seagrass area in Brgy. Bolitoc, Sta. Cruz, Zambales was contaminated with nickel which may have been caused by its location adjacent to the stockpiles.

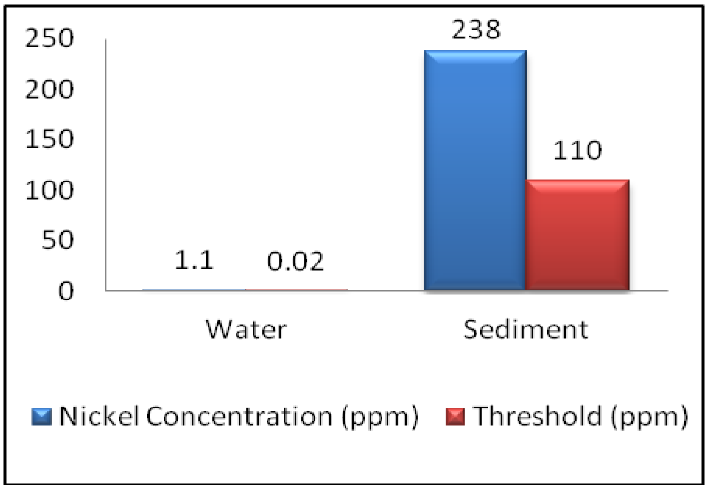


Fig. 2 Nickel concentration in the water and sediment samples in the sea grass ecosystem

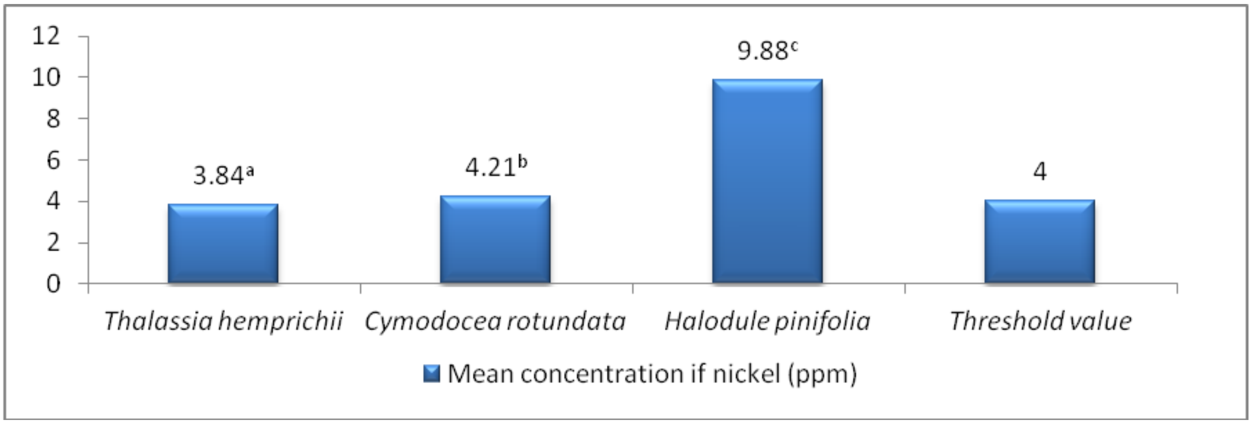
Sea grass samples Ni concentration

Sea grasses are the second line of defense of the marine resources against sedimentation/siltation next to the mangroves. Sea grasses like any other plants require nutrients and minerals from their surroundings. These underwater flowering plants absorb these essential compounds from the soil making these potential aides for lessening the pollution in certain contaminated sites such as the ocean, specifically the coastal area. Sea grass species in the coastal area of Brgy. Bolitoc were collected for nickel analysis. Whole plants of *Thalassia hemprichii*, *Cymodocea rotundata* and *Halodule pinifolia* were collected to determine which among them can accumulate nickel from their environment.

Figure 3 illustrates the variation between the mean concentrations of nickel for the three species of seagrasses and the threshold value of nickel. As shown, nickel concentrations in sea grass plant tissues ranged from 3.84 ppm to 9.88 ppm. Results imply significant difference among the means in which *Halodule pinifolia* obtained the highest nickel concentration of 9.88 ppm, followed by *Cymodocea rotundata* (4.21ppm) and *Thalassia hemprichii* (3.84 ppm) which are not significantly different from each other. The seagrass species exceeded the average nickel concentration in plants, except *T. hemrprichii*. d

Seagrasses have moderate capability to uptake heavy metals from their environment [22]. A study in China suggested that *Thalassia hemprichii*, Enhalus acoroides and *Cymodocea rotundata* were able to accumulate heavy metals such as cadmium, supported by a study which observed that *H. pinifolia* showed copper, zinc and lead in their tissues [23, 16]. The result in the tissues of *H. pinifolia* is nickel accumulation in three different sites. Moreover, another study also reported heavy metal (Mn, Zn and Fe) absorption in the tissues of *H. pinifolia* [19]. Furthermore, *H. pinifolia* was described to be a strong heavy metal accumulator, third in line from the other two Halodule species observed in Lakshadweep group of Islands [23].

The presence of nickel in *C. rotundata* is supported by a study that posited a species under genus *Cymodocea* accumulated nickel in its tissues [22]. The nickel accumulation activity of *C. rorundata*, was also confirmed because like *H. pinifolia*, this species also showed the capacity to absorb nickel in the three different sites of the study [23]. On the other hand, *C. rotundata* was also observed to have the capability to phytoremediate other metals such as copper



Small letters indicate the difference of nickel contents when compared between each plant species and the threshold value (LSD: $p < 0.05$).

Fig. 3 Mean concentration (ppm) of nickel in the seagrass species

and lead in its tissues [19,18].

Nickel concentration in the tissues of *T. hemrprichii* was also confirmed from another experiment showing that the seagrass species accumulated nickel in the two study sites in which it occurred [23]. In addition, *T. hemrprichii* was also able to absorb sodium and calcium duringscientific observations in the seagrasses of Gulf of Mannar, India [19]. Another study reported that *T. hemrprichii*, along with other species of seagrasses and seaweeds, could take up heavy metals including nickel in its tissues [7]. Furthermore, it was generalized in that seagrasses with small leaves, such as the three *Halodule* species, are more efficient in heavy metal uptake compared to species of seagrasses with larger leaves [23], and it was also concluded that seagrasses are good biomonitoring tools and heavy metal sink, since the biomass take usually long term to mineralize in nature.

Seaweed

Ni Contamination in seaweed area

Water and sediment samples were subjected for analysis using FAAS to measure the level of nickel concentration in the area of the seaweed site. Figure 4 shows that for the water sample, nickel concentration was at 1.0ppm and higher when compared to the threshold value of nickel for water at 0.02ppm. On the other hand, the the level of nickel in sediment sample reached 92ppm, yet it was still at normal range since it did not exceed the threshold limit of nickel for sediment. The concentration of nickel in both matrices was highly influenced by the proximity of the area from the stockpile and port of the nickel mining companies. There are three probable reasons that resulted to the accumulation of nickel on the sediment and water can be enumerated: 1) there is a high chance that during rainy season the nickel dusts run-off towards the sea; 2) during dry, windy season the dusts were transferred to the sea; and 3) during the

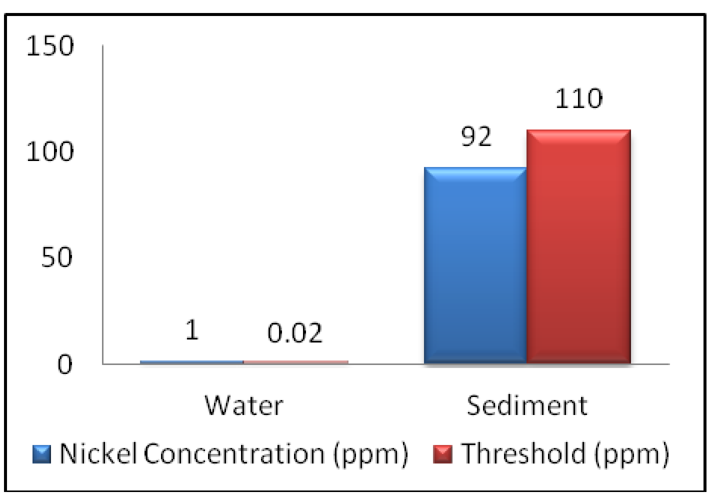
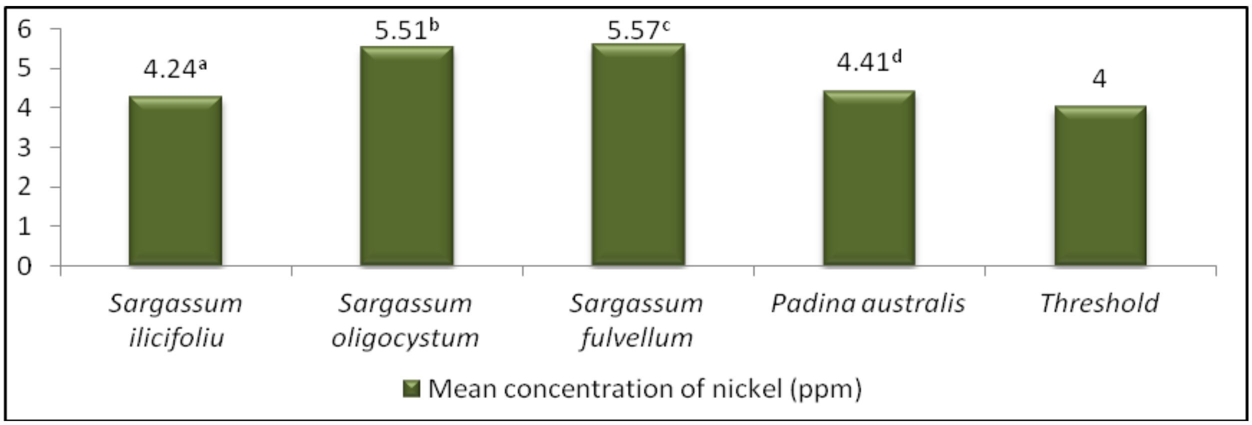


Figure 4. Nickel concentration in the water and sediment samples in the seaweed ecosystem.

transfer of nickel laterites to transporting vessels at the port, some may have been dumped directly to the sea.

Nickel Analysis and Phytoremediation Potential of Seaweed Species

Some sea weeds are mainly utilized as food while some are used as fertilizers. However, metal uptake capability of seaweeds is the concern of this study. The four species of seaweeds from Bgry. Bolitoc wereanalyzed for their phytoremediation capability for nickel. Figure 4 presents the mean concentration of nickel in each seaweed species from the study site. Results show that the mean nickel contents of the four seaweed species from the seaweed site of Brgy. Bolitoc, Sta. Cruz, Zambales ranged from 4.24 ppm to 5.57 ppm. Results show that among the seaweeds, *S. fulvellum* (5.57 ppm) and *S. oligocystum* (5.51 ppm) accumulated almost the same amount of nickel while the other two species also had same amount of nickel absorption, *S. ilicifolium* (4.41 ppm) and *Padina australis* (4.24ppm). As shown in Table 4, all seaweed species exceeded the normal range of nickel in plant tissues which implies that these seaweeds have tolerance for nickel contamination in the area, and have the capability to absorb nickel in their tissues. Results indicate that seaweed species present in the seaweed site are capable of phytoremediation. Nickel was found present in *S. ilicifolium*; this result is supported by a study which observed the accumulation of nickel in the stem of this species [14].



Small letters indicate the difference of nickel contents when compared between each plant species and the threshold value (LSD: $p < 0.05$).

Fig. 5 Mean concentration (ppm) of nickel in the seaweed species

In addition, *Sargassum* sp. and *Padina* sp. had the highest biosorption compared to other seaweeds observed in one study [21]. Moreover, another study reported that under the same genus of *Padina, P. gymnospora* took up high levels of cadmium, manganese and nickel [2]. Under the genus *Sargassum*, other seaweed species were reported to have absorbed nickel in their tissues; this includes *S. wightii* and *S. fluitans*, [22,20,14]. Other *Sargassum sp*. which can accumulate nickel are *S. liniarifolium* and *S. polycystum* [14]. Moreover, other *Sargassum sp*. are also capable to accumulate other heavy metal such as copper, iron, zinc and lead [5,6,10]. Seaweeds are excellent means of filtration for heavy metals such as zinc, cadmium, copper, nickel and iron and some potential carcinogens dissolved in seawater [22]. Efficiency in metal uptake by brown algae is due to the high levels of sulphated polysaccharides and alginates present in the cell walls of these species to which metals are being attracted to [3]. Nonetheless, Sargassum species present in the coastal areas of Brgy. Bolitoc absorb nickel and can help lessen contamination of water.

CONCLUSION

Analysis of water and sediment samples from the two ecosystems concludes that the area are indeed contaminated with nickel. Moreover, seven marine species such as three seagrass species, *Cymodocea rotundata*, *Thalassia hemprichii* and *Halodule pinifolia* and four seaweed species, *Sargassum fulvellum*, *Sargassum ilicifolium*, *Sargassum olicystum* and *Padina australis*, showed capability to absorb nickel from their surroundings. These seagrass and seaweed species are potential phytoremediator. These species may help restore the once pristine status of Brgy. Bolitoc, Sta. Cruz, Zambales, hence, their conservation is very imperative.

RECOMMENDATION

Based on the results of this study, phytoremediation assessment of the seaweeds in the area should be performed once again during summer months. Moreover, an ex-situ experiment on phytoremediation using the seaweeds in this study may be performed to further assess their capability to absorb nickel. Conservation, restoration and management of seagrass species are recommended to help the recovery of these marine plants from deterioration. Also, the wastewater from the residential area as well as the nickel laterites from the stockpile near the study site should be addressed since these are the probable cause of the species' lowering population. Restoration of the seagrass bed in Brgy. Bolitoc, Sta. Cruz Zambales will help resolve the problem of nickel contamination in the area.

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