RESEARCH COMMUNICATION

Ferronickel slag toxicity tests on *Chlorella vulgaris* and *Artemia* sp.

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Abstract

Acute effects of ferronickel slag toxicity on *Chlorella vulgaris* and *Artemia* sp. were studied. Tests were conducted on ferronickel slag to determine the concentration of heavy metals leached out to the environment. Toxicity tests were also carried out on the organisms with minimum exposure duration of 4 days or until the occurrence of a negative effect. About 400 cells mL^{-1} of *C. vulgaris* and 20 individuals of *Artemia* sp. were used in each of the reactors with media containing slag concentration ranged from 0 to 50%. Results showed that the IC50 (inhibition concentration) value of the percentage of slag (w/v) for *C. vulgaris* was 5-10%. Slag toxicity test on *Artemia* showed that LC50 (lethal concentration) for the percentage of slag was also between 5-10%. The study proved beyond doubt the acute effects of the slag at low concentration (10% w/v) as indicated by the inhibition of growth of 60% of the *C. vulgaris* population and deaths of more than 50% of the *Artemia* in the reactors. Hence the study suggests wise use of the slag to avoid disturbances to environment and society at large.

Keywords: *Artemia*; *Chlorella vulgaris*; ferronickel slag; heavy metal; toxicity

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Introduction

Slag is one of the by-products of nickel seed processing industry which ultimately converted to ferronickel (Ingots & Shot). The slag produced in such processes can be as high as 50-75% (Svana & Ysteb, 1990). Ferronickel processing industry in Illigan City, Philippines produces up to 500,000 metric tons of slag per year (Demotica, Amparado Jr., Malaluan, & Demayo, 2012). The slag has been used extensively in some countries possibly due to the omission of the same from the hazardous waste classification. In Illigan Bay, slag is widely being used as a material for reclamation. In the Illawarra and Newcastle, Australia, the slag has been applied as a substitute for cement for several years (Julli, 1999; Demotica *et al*., 2012).

Indonesian Governmental Regulation No.18 and 85 (1999) regarding hazardous and toxic waste management mentions the slag from the processing of nickel under hazardous waste category. It is due to the presence of high concentrations of heavy metals that are harmful to health of human beings and other organisms (Kozanoglou & Catsiki, 1997; Tsangaris, Papathanasiou, & Cotou, 2007; Catsiki, Bel, & Nicolaidou, 1994). There are several ferronickel industries that will be built in Indonesia. But before commencing any utilization programme, study of the effect of slag on the environment is necessary. Toxicity tests on aquatic marine organisms such as *C. vulgaris* (single-cell green algae, belonging to the phylum Chlorophyta) and *Artemia* sp. (aquatic crustaceans known as brine shrimp belongs to the family Artemiidae) will give an idea on the effect of slag on sea environment (Dinesh *et al*., 2014; Long & MacDonald, 1998; Smith, Savino, & Blouin, 1988). In contrast to other studies aimed at understanding toxicity effects of ferronickel slag on any particular group of organisms, the present study aimed at analyzing the same simultaneously on representative plant and animal systems.

Material and methods

Toxic Characteristic Leaching Procedure (TCLP) test (US EPA, 1992) was conducted on ferronickel slag to determine the leaching of heavy metals that exceed the quality standard. It is a soil sample extraction method for chemical analysis employed as an analytical method to simulate leaching through a landfill. The testing methodology is used to determine if a waste is characteristically hazardous or not. The toxicity of ferronickel slag on *C. vulgaris* and *Artemia* sp. that have been acclimatized previously was conducted where minimum duration of exposure was 4 days or until the occurrence of a negative effect on the organisms. Concentrations (%) of slag used in the study include 0 (designated as C0), 2 (C1), 5 (C2), 10 (C3), 20 (C4), and 50 (C5). Approximately 400 cells ml^{-1} for *C. vulgaris* and 20 individuals of *Artemia* sp. were used in each reactor. Observation of the number of dead organisms was carried out every day and determined no observed effect concentration (NOEC), lowest observed effect concentration (LOEC), and the IC50 (inhibition concentration) for *C. vulgaris* and LC50 (lethal concentration) for *Artemia* sp.

Results and discussion

Analysis of TCLP test results

The TCLP test was conducted on the slag for heavy metals such as Fe, Cr, Cu, Pb, and Cd (Table 1). The TCLP test indicated that content of Cr exceeded the quality standard based on Governmental Regulation No.18/1999, which states 0.25 ppm as the maximum levels of Cr in the waste materials.

Table 1. Comparison of slag TCLP test results and quality standards (Governmental Regulation No.18/1999)

Heavy Metals	Slag	Quality standards
Fe	1586775	NL
Cr	0.645	0.25
Cu	ND	0.19
Pb	0.0219	2.50
Cd	ND	0.05

Quantity of the heavy metals are expressed in PPM.
ND = Not detected; NL = Not listed.

Toxicity of ferronickel slag on *C. vulgaris*

Results of acute toxicity tests on *C. vulgaris* showed that the slag affected the growth of the organism (Fig. 1a). The results clearly showed that the cell density of *C. vulgaris* differ considerably among controls and test samples with varying percentage of slag. The density difference of *C. vulgaris* was observed since the 2nd day of the experiment. However, almost same density levels were noticed for slag content ranging from 2 to 20%. It was clearly noticed that density of *C. vulgaris* at 50% of the slag content was significantly lower than that of any other percentage range. This is consistent with the study of Qian *et al*. (2013) which stated that exposure to Cr ions can inhibit the growth of *C. vulgaris*.

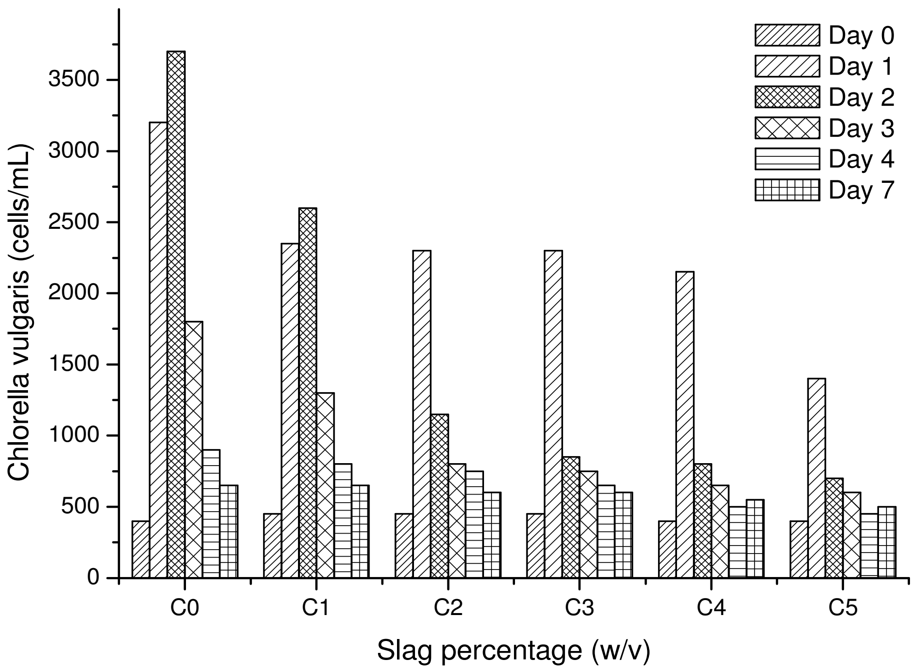
Exposure to slag affected the density of *C. vulgaris* in the media. Difference in cell density of *C. vulgaris* between the beginning and the last observation day was counted as yield and the difference in percentage of yield between the treatment and control of various slag percentages was calculated as the percent inhibition. The slag toxicity tests determined that the IC50 of the percentage of slag (w/v) on *C. vulgaris* was 5-10% (Fig. 1b). Tests conducted at slag percentages of 2, 5, 10, 20, and 50 (w/v) resulted into percent inhibition of 30, 40, 60, 80, and 90 respectively.

Based on the toxicity tests conducted, it was observed that exposure to slag at higher concentration inhibits the growth of *C. vulgaris*. It is because of the heavy metals, especially Cr, present in the slag (Table 1) which exceeded that of the quality standards based on Government Regulation No. 18 of 1999. The Cr is absorbed into the algal cells which disrupt chloroplast structure and reduce the levels of chlorophyll. Besides, Cr exposure also can increase the volume of the vacuole that reduces the space for the chloroplast. Eventually, due to reduced chlorophyll content, photosynthesis is inhibited and therefore contributes to reduced growth of *C. vulgaris* (D'ors, Pereira, Bartolome, Lopez-Rodas, Costas, & Sanchez-Fortun, 2010). In addition, exposure to Cr ions can also cause structural changes in the cell nucleus that affect the growth of algal symbionts (Licursi and Gomez, 2013).

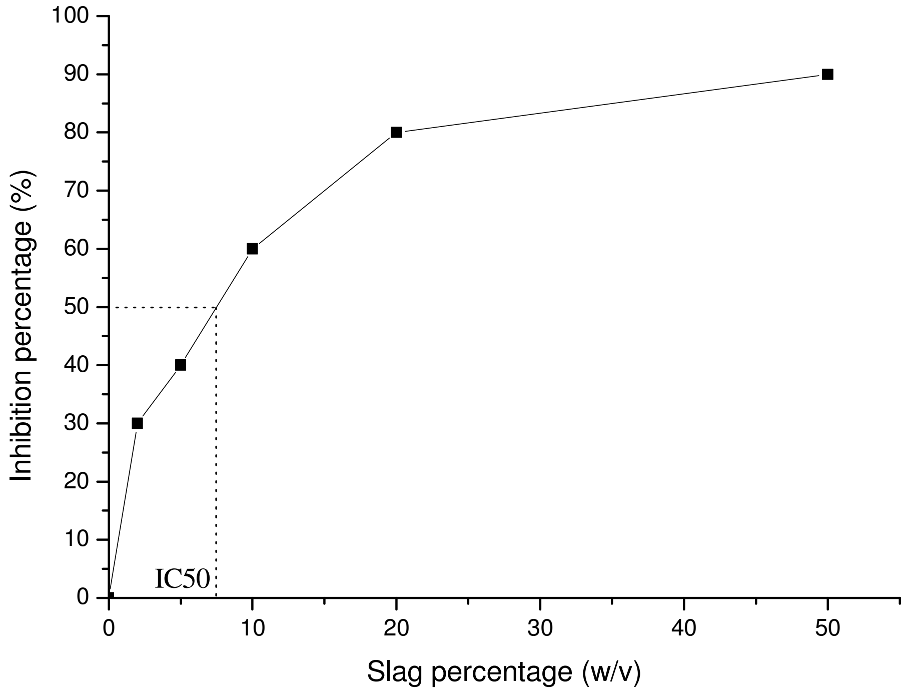
There are differences in the Cr content in the media with and without slag which was used to grow *C. vulgaris*. It was noted that the higher the percentage of slag, Cr content in aqueous media also tends to increase due to leaching (Fig. 2). However after growth, the Cr content in the water was lower than medium without *C. vulgaris*. This suggests that the Cr leached out from the slag into the media entered into the *C. vulgaris* system and accumulated there so that Cr content in the aqueous media with *C. vulgaris* was reduced considerably (Fig. 2). The presence of Cr inside the cell of *C. vulgaris* can cause negative effects, such as reduced rate of photosynthesis and growth disorders.

Toxicity of ferronickel slag on *Artemia* sp.

The slag toxicity tests determined that the LC50 of the percentage of slag (w/v) on *Artemia* sp. was 5-10% (Fig. 3a and Fig. 3b). When the slag concentration was higher than 10%, death of more than 50% of the population of *Artemia* in the reactor was noted. The death of *Artemia* might be due to acute toxic effects of heavy metals from slag, especially Cr because based on the TCLP test results of the



(a)



(b)

Fig. 1. Comparison of the effect of slag concentration (w/v) on *C. vulgaris* (a) and the relationship between the percentage of slag (w/v) with the percent inhibition (b).

slag (Table 1), the Cr content of the slag exceeded the quality standards.

There was difference in Cr content in the media with and without *Artemia*. In the graph (Fig. 4) it can be seen that the higher the percentage of slag, Cr content in aqueous media also tends to increase because the process

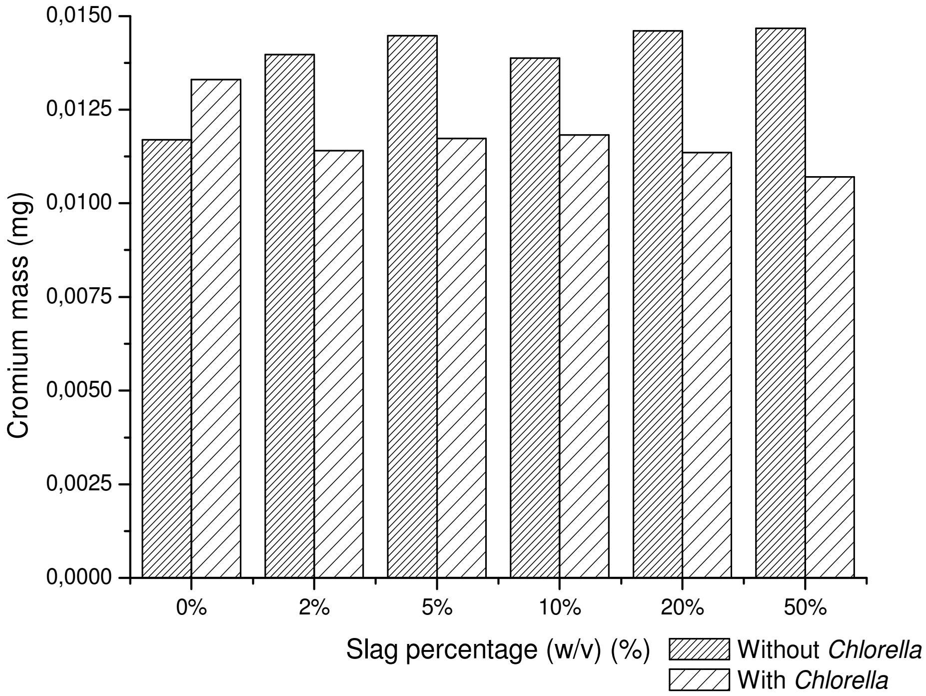


Fig. 2. Comparison of chromium content in medium with and without *C. vulgaris*.

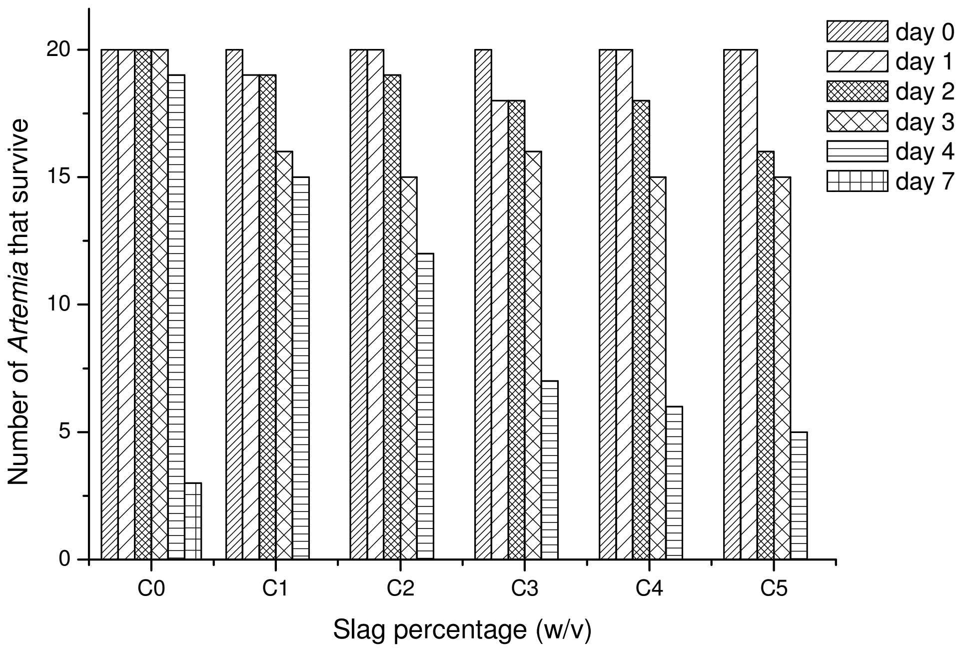


Fig. 3a. Comparison of the effect of slag concentration (w/v) on *Artemia*

of leaching as noted for *C. vulgaris*. Comparatively, the Cr content was lower in aqueous media with *Artemia*. This indicates that the Cr entered into the body of *Artemia* from the water so that the mass of Cr on media with *Artemia* was reduced. The presence of heavy metal Cr inside the body of *Artemia* can cause negative effects including mortality.

Exposure to Cr can influence on the survival of population of *Artemia* which is very sensitive to pollutants. Studies (Verriopoulos, Milliou, & Moraitou-Apastolopoulou, 1988) found that acute exposure to Cr can affect physiology of *Artemia*, mainly

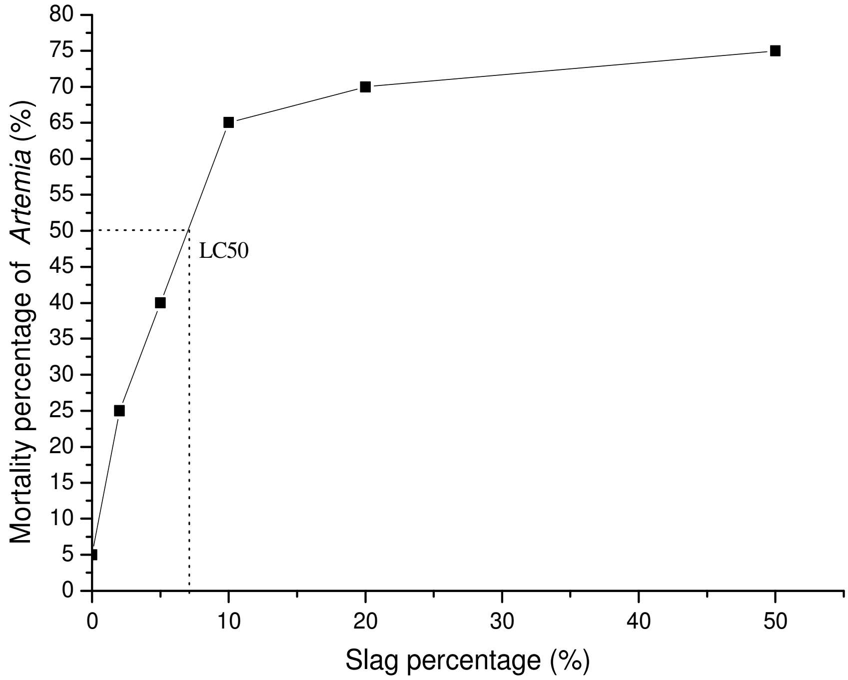


Fig. 3b. The relationship between the percentage of slag (w/v) and percentage mortality *of Artemia*

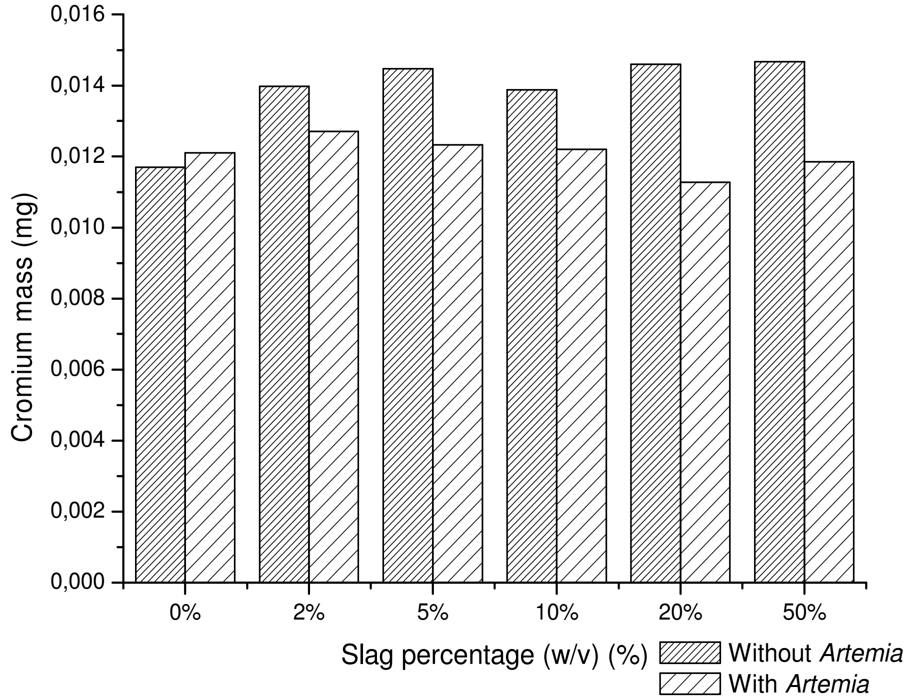


Fig. 4. Comparison of chromium content in medium with and without *Artemia*

with an increase in the intensity of respiration. In addition, Cr can also cause destructive oxidative stress that can lead to death of *Artemia* (Li *et al*., 2013; Li, Z-H., Li, P., & Randak, 2011).

Conclusions

The present study found out that the slag was toxic even at lower concentrations on *C. vulgaris* as inferred from the

IC50 which was 5-10% (w/v). Besides, the slag toxicity test on *Artemia* showed that the LC50 was also between 5-10%. Acute effects of slag on *Artemia* was noted at 10% of slag (w/v) indicated by the deaths of more than 50% of the population of *Artemia* in the reactor. The treatments caused a dose dependant inhibition of growth. The study clearly showed the effect of slag on marine organisms.

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