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Dried and Wet *Trichoderma* sp. Biomass Adsorption Capacity on Ni, Cd and Cr in Contaminated Groundwater

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Abstract. Groundwater sampled from five different boreholes at a decommissioned landfill site show contamination with heavy metals Cd, Ni and Cr. The nickel level ranges from 0.10 to 0.321 mg/L, cadmium levels ranges from 0.051 to 0.184 mg/L and chromium having concentrations from 0.027 to 0.122 mg/L. The assessment of the metal-binding capacity of dried and wet fungal biomass (*Trichoderma* sp.*)* on the contaminated groundwater is studied. Results show that 2.0 g dried biomass of *Trichoderma* sp. could remove 100 percent Cd, Cr and Ni in the groundwater after 48 contact hours. This is more effective than 20.0g wet biomass, achieving 98 percent removals during the same period. Dried biomass has greater efficiency in the adsorption of all metals from the 5 sources tested. Of the three metals, cadmium showed greater affinity to the biomass, followed by nickel and chromium. The adsorption rate is most rapid the first 24 hours for all metals and is independent of the original concentrations of the metals. The Fourier Transformation Infrared analysis (FTIR) conducted shows *Trichoderma* sp. possesses functional groups such as amino and hydroxyl groups and these groups influenced the adsorption of heavy metals.

Keywords: Heavy metals, *Trichoderma* sp., FTIR, adsorption, groundwater contamination

1. Introduction

Heavy metal pollution is one of the major environmental problems in the current society. The kinds of heavy metals of major concerns include toxic metals such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, and Sn, precious metals, Pd, Pt, Ag, Au, Ru and radionuclides like U, Th, Ra and Rn [19]. The polluted wastewaters containing these heavy metals will enter streams and affect the environment adversely [2]. Conventional methods commonly used to remove dissolved heavy metals include chemical precipitation, sludge separation, chemical oxidation and reduction, ion exchange, reverse osmosis, filtration, adsorption using activated charcoal, electrochemical treatment and evaporative recovery [3, 7, 17]. All of these methods are expensive and may not always be feasible. Besides that, bacteria, fungi, algae, mosses, macrophytes and higher plants can remove heavy metals too [9, 10, 13]. However, fungal biomass such as fungi is being considered more and is one of the alternative methods that is cheaper compared to others [17] The use of biomass of fungi [12], algae and bacteria [5] for removal of heavy metals from aqueous solutions is gaining increasing attention. According to [15] it has been found that both living and dead microbial cells adsorb metal ions. The biomass can bind heavy metals either actively or passively or by combination of both processes [6]. Bioadsorption can be accomplished by simple physical methods without damaging the biosorbent's structural integrity and accomplish a high efficiency in the removal of heavy metals in aqueous solution. This study aims to investigate the biosorption capacity of *Trichoderma* sp. on nickel, cadmium and chromium in groundwater from landfill. The FTIR results were analyzed and functional groups before and after treatment in the FTIR peaks were determined.

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2. Materials and method

For experiment purposes the groundwater sampling was carried out from five existing boreholes located at a decommissioned sanitary landfill site at Seberang Perai Selatan, Penang, Malaysia. The landfill was built based on the Fukuoka design of Japan. The sampling was carried out after the water in the borehole well was allowed to stabilize to static water level prior to water level measurements for at least 7 days. Water level was documented and purging was done for at least 2 or 3 wells volumes. Later, the water level in each well was allowed to recover to its pre-purge static water level then only collection of the groundwater was conducted within 24 hours using clean disposable bailer and clean sample container following standard method and procedure of APHA. The samples were transported to the laboratory in ice and stored at 4ºC and for treatment purposes.

The groundwater was analyzed for the presence of all metals using atomic absorption spectrophotometer (Perkin Elmer model A. Analyst 200). All measurements were carried out in an air/acetylene flame. Determination of the three highest metals was made and applied for the succeeding treatment experiments. The three highest metal ions, Ni, Cd and Cr present before and after biosorption experiments were also analyzed using the same AAS unit. Each experiment was repeated three times and the results given are the average values. The deviation is less than 5%.

Solid agar media was prepared using 160ml of mixed vegetable juice with 90 ml of distilled water and 3g of Nutrient Agar, mixed thoroughly and adjusted to pH 5.01. The solution is then autoclaved for 121°C, 20psi for 15minutes. Liquid media was made up of 30% mixed vegetable juice, with pH 5.01 and poured into 100 ml Erlenmeyer flask and autoclaved for 121°C, 20 psi for 15minutes. Both the solid and liquid media were allowed to cool to room temperature before use. *Trichoderma* strains were cultured in solid media. Additionally, the liquid media were then inoculated with fresh mycelia plug from solid media. The submerged fungal culture is allowed to grow for five days under 12 hours white light and 24^{o} Celcius before use for further treatment.

As for treatment test the groundwater samples taken from the five boreholes were determined for Ni, Cd and Cr concentrations. Two types of *Trichoderma* biomass were used, wet and dried biomass. The wet biomass of *Trichoderma* sp. was harvested by filtering the liquid fungal culture through Whatman filter paper with pore size of 45 µm. The biomass was washed with deionized water several times. After washing, the biomass was weighed to get the wet weight. A portion of the wet biomass was freeze dried using freeze dryer model LABCONCO and ground to 250 µm using grinder (IKA model A11 basic). This constitutes dry mass. Preliminary works indicated that 20 g wet biomass and 2.0 g dry biomass work effectively for the treatment study. Hence, the treatments were carried out using 20g and 2.0g each of the wet and dry mass and placed in clean plastic bottles together with 150 ml of sampled contaminated groundwater. The treated bottles were incubated on a rotary shaker at 160 rpm, at room temperature and aliquots of the groundwater or filtrate were taken out for analysis every 24, 48 and 76 hours. Finally, the filtrates were analyzed using Atomic Absorption Spectrophotometer (AAS) for heavy metal content. At the same time, the fungal culture at the end of each incubation period was filtered out and analyzed using FTIR.

3. Results and discussion

3.1. Culturing of *Trichoderma* sp.

The *Trichoderma sp.* were obtained from Pro-Fil Industrial Resources Sdn. Bhd., Penang, Malaysia and isolates were selected for further studies based on the fungi ability to adsorb metal ions. The fungal detailed structural morphologies observed under scanning electron microscope as in Fig. 1, is characterized by the presence of septate mycelia and its conidiophores are branches consisting group 3 or 4 philiades which is swollen in the middle and narrow at the ends. Its conidia are ellipsoidal in shape and bear by philiades.

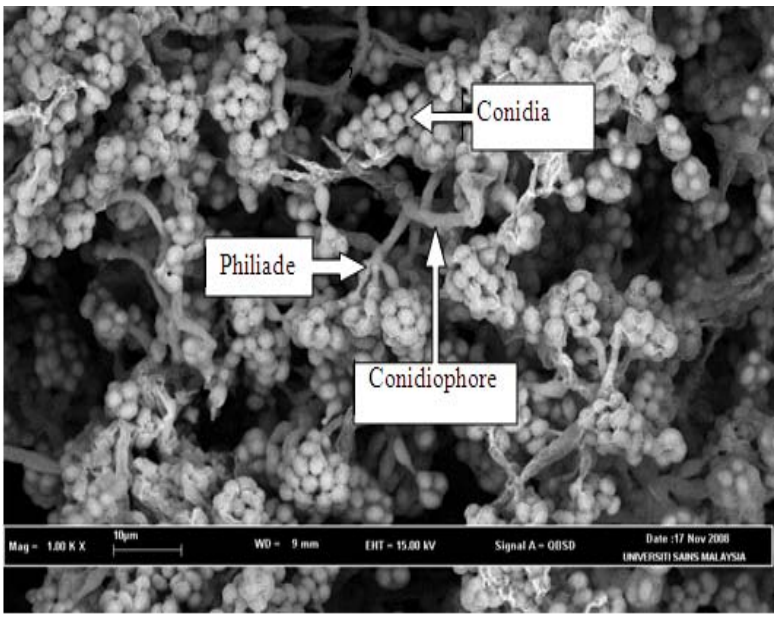

Fig. 1: *Trichoderma* sp. morphological structure under Scanning Electron Microscope (1000 × magnification).

3.2. Groundwater analysis

Groundwater sampled from the boreholes showed nickel level are greater than 0.10 mg/L. Fig. 2 shows nickel concentrations to be 0.100 mg/L, 0.198 mg/L, 0.321 mg/L, 0.153 mg/L and 0.136 mg/L. Cadmium levels ranged from 0.051 to 0.184 mg/L and chromium having concentrations in the groundwater from 0.027 to 0.122 mg/L. It appears that highest contamination with heavy metals occurred at borehole 3 which is located at the lowest elevation.

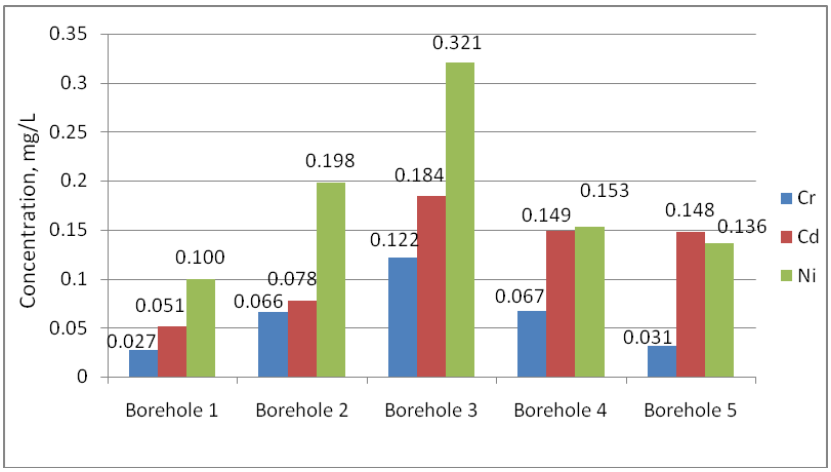

Fig. 2: Concentrations of heavy metals(Cr, Cd and Ni) in groundwater from five different boreholes

3.3. Treatment

The treatments were carried out using 20g and 2.0g each of the wet and dry mass of *Trichoderma sp.* placed in clean plastic bottles together with 150 ml of sampled contaminated groundwater.

The results in Table 1 shows adsorption of heavy metals took effect within 24 hours of treatment with dried fungal biomass. Percent nickel removal after 24 hours from the five boreholes or sampling sources varies from 77% to 89.41%. The variation also occurs between heavy metals from the same source of the groundwater. At 24 hours, more than 84% cadmium in the groundwater from borehole 1 have been adsorbed onto the biomass, which is higher than chromium at about 81.5% and nickel at about 77%.

By 48 hours all the cadmium and chromium from the groundwater borehole 1 have been removed, leaving 0.002mg/L nickel in the water. Similar observations were made for samples taken from all the other boreholes. Removals of heavy metals using dried fungal biomass can be achieved in about 48 hours contact time. Rate of heavy metal removals differ based on the source, in this case, different boreholes, of the

contaminated groundwater. It however appears to be independent of the original concentration in the sample, proven by the different percent uptake pattern from the different boreholes which did not reflect adsorption to be concentration dependent. Of the three heavy metals, cadmium seems to have greater affinity to the fungal dried biomass, with the fastest uptake, followed by nickel and chromium. Given enough contact time, all the heavy metals are being removed from the groundwater by the biomass. The initial 24 hour removals of the heavy metals from different sources show lower adsorption by wet or live biomass than dried or dead biomass. However, at 76 hour contact time, almost all the heavy metals are being removed from the water, indicating that either wet or dried fungal biomass, can be used in the biosorption of nickel, cadmium and chromium from contaminated groundwater. The dried biomass is metabolically inactive, but possesses larger surface area. The heavy-metal ions can be adsorbed onto the functional groups present on the surfaces of the dried biomass. The metal ions can also be absorbed onto the binding sites present in the cellular structure.

According to [8], the metal binding capacity of the *Trichoderma* biomass permits the removal of toxic metals from contaminated water through biosorption onto dead and inactive fungal biomass. The adsorption can also take place on live and active biomass through metabolically mediated or physico-chemical pathways of uptake and allows the accumulation of heavy metals into the cells. The morphological differences within the biomass (dead or alive) can greatly influence the biosorption process. The live cellular structure of *Trichoderma* takes up metal ions rather than adsorbed which can make significant difference in metal uptake between dry and wet biomass. *Trichoderma* as a biosorbent adsorb Cd quicker than Ni and Cr, however there is no distinct selectivity among Ni, Cr and Cd, binding. The presence of other metal ions in the groundwater did not show competitive behaviour of the metals to attach to the active sites, indicating the amount of the biomass used was not a limiting factor.

Table1: Reduction in nickel concentrations in contaminated groundwater from landfill after treatment with 2.0 mg *Trichoderma* sp. dry biomass.

	Nickel						
		24 hours		48 hours		76 hours	
Borehole	Initial conc, (mg/L)	Final conc, (mg/L)	%Removal	Final conc (mg/L)	%Removal	Final conc, (mg/L)	
1	0.100	0.023	77.00	0.002	98.00	-	100.00
2	0.198	0.031	84.34	0.007	96.46	-	100.00
3	0.321	0.034	89.41	0.010	96.88	-	100.00
4	0.153	0.025	83.66	0.004	97.39	-	100.00
5	0.136	0.024	82.35	0.003	97.79	-	100.00

Table 2: Reduction in cadmium concentrations in contaminated groundwater from landfill after treatment with 2.0 mg *Trichoderma* sp. dry biomass

	Cadmium						
		24 hours		48 hours		76 hours	
Borehole	Initial conc, (mg/L)	Final conc, (mg/L)	%Removal	Final conc (mg/L)	%Removal	Final conc, (mg/L)	%Removal
1	0.051	0.008	84.31	-	100.00	-	100.00
2	0.078	0.011	85.90	-	100.00	-	100.00
3	0.184	0.015	91.85	0.001	99.50	-	100.00
4	0.149	0.011	92.62	-	100.00	-	100.00
5	0.148	0.009	93.92	-	100.00	-	100.00

Table 3: Reduction in chromium concentrations in contaminated groundwater from landfill after treatment with 2.0 mg

Trichoderma sp. dry biomass.

	Chromium						
		24 hours		48 hours		76 hours	
Borehole	Initial conc, (mg/L)	Final conc, (mg/L)	%Removal	Final conc (mg/L)	%Removal	Final conc, (mg/L)	%Removal
1	0.027	0.005	81.48	-	100.00	-	100.00
2	0.066	0.011	83.33	-	100.00	-	100.00
3	0.122	0.029	76.23	0.009	92.62	-	100.00
4	0.067	0.016	76.12	0.003	95.52	-	100.00
5	0.031	0.008	74.19	-	100.00	-	100.00

Table 4: Reduction in nickel concentrations in contaminated groundwater from landfill after treatment with 20.0 mg *Trichoderma* sp. wet biomass

	Nickel						
		24 hours		48 hours		76 hours	
Borehole	Initial conc, (mg/L)	Final conc, (mg/L)	%Removal	Final conc (mg/L)	%Removal	Final conc, (mg/L)	%Removal
1	0.100	0.035	65.00	0.006	94.00	-	100.00
2	0.198	0.043	78.28	0.012	93.94	-	100.00
3	0.321	0.054	83.18	0.021	93.46	0.002	99.38
4	0.153	0.035	77.12	0.010	93.46	-	100.00
5	0.136	0.037	72.79	0.009	93.38	-	100.00

Table 5: Reduction in cadmium concentrations in contaminated groundwater from landfill after treatment with 20.0 mg *Trichoderma* sp. wet biomass

	Cadmium						
		24 hours		48 hours		76 hours	
Borehole	Initial conc, (mg/L)	Final conc, (mg/L)	%Removal	Final conc (mg/L)	%Removal	Final conc, (mg/L)	%Removal
1	0.051	0.017	66.67	0.004	92.16	-	100.00
2	0.078	0.022	71.79	0.004	94.87	-	100.00
3	0.184	0.036	80.43	0.007	96.20	-	100.00
4	0.149	0.029	80.54	0.006	95.97	-	100.00
5	0.148	0.024	83.78	0.005	96.62	-	100.00

Table 6: Reduction in chromium concentrations in contaminated groundwater from landfill after treatment with 20.0 mg *Trichoderma* sp. wet biomass

	Chromium						
		24 hours		48 hours		76 hours	
Borehole	Initial conc, (mg/L)	Final conc, (mg/L)	%Removal	Final conc (mg/L)	%Removal	Final conc, (mg/L)	%Removal
1	0.027	0.008	70.37	-	100.00	-	100.00
2	0.066	0.020	69.70	0.005	81.48	-	100.00
3	0.122	0.031	72.95	0.012	81.81	-	100.00
4	0.067	0.022	67.16	0.009	92.62	-	100.00

5	0.031	0.011	64.52	0.002	93.55	-	100.00

3.4. FTIR spectral analysis

Strong adsorption behaviour of certain micro-organisms towards metal ions is a function of the chemical make-up of the biomass. The Fourier transform infrared (FT-IR) study of the *Trichoderma* sp. is needed to know the chemical make up that played a role in the adsorption of metal. Major characteristic bands recorded included: a broad O-H and N-H stretching vibration band obtained around 3413 cm^{-1} for intermolecular hydrogen bonding (H-bonded OH groups) attributed to phenolic groups. Methylene hydrogens (-CH2-) gave rise to two aliphatic C-H stretching bands; a sharp peak at 2925 $cm^{-1}$for asymmetric stretching and a shoulder at 2852 cm^{-1} for symmetric stretching. A pronounced peak at 1652 cm^{-1} could be attributed to aromatic C=C, C=O and/or C=O of conjugated ketones or to C=N amide stretching. The methyl asymmetric C-H bending was observed at 1458 and 1053 and 1034 cm^{-1} could be attributed to C-O stretching of alcohol, sulfoxides, carbohydrates or polysaccharides-like substances. The results concurred with [11] who found in *T. harzianum* the presence of OH, =CH and C=O and NH_2 functional groups. Little changes in the peak positions in the spectrum of the *Trichoderma* sp. indicates the binding of nickel, cadmium and chromium with amino and hydroxyl groups. Many of the characteristic bands are shifted at those maximum peaks, and absorbance is changed due to the effects of the heavy metal. This can be shown in all the FTIR spectra of *Trichoderma* sp. treated with groundwater contaminated with heavy metals as in Fig. 3. It is reasonable to assume, based on the change of the band, that the peak values suggested metal chelating. The structure of the metal bound to carboxyl ligands on the fungus is likely to take place.

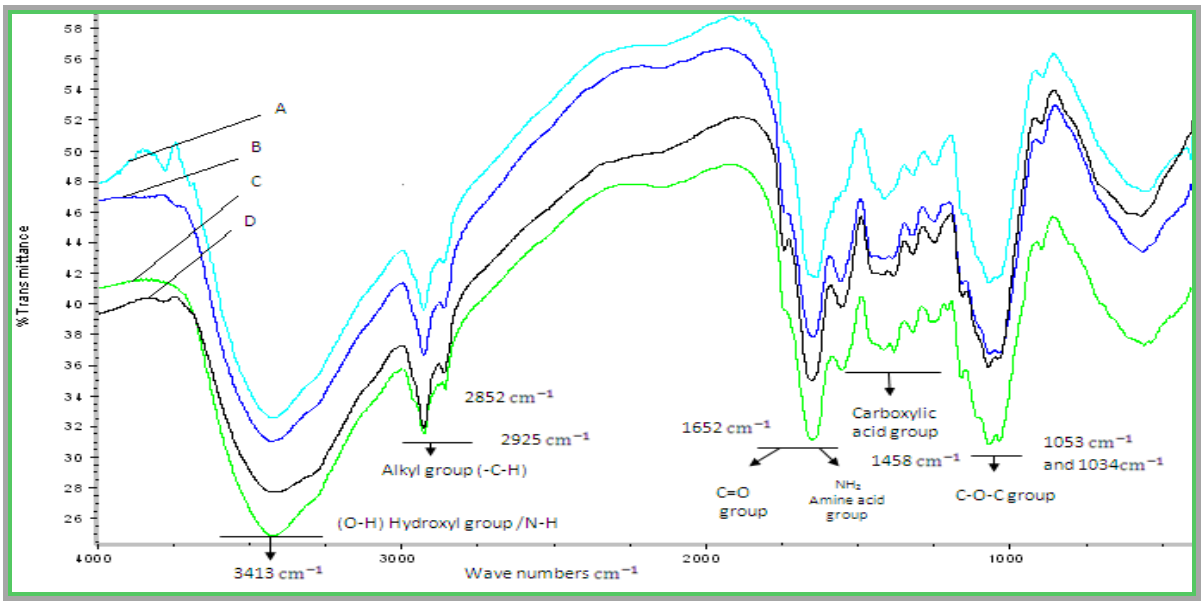

Fig. 3: FTIR spectra of Trichoderma sp. A: *Trichoderma* sp. before treatment; B : *Trichoderma* sp. after treated with Nickel; C : *Trichoderma* sp. after treated with Chromium; D : *Trichoderma* sp. after treated with Cadmium.

The most remarkable difference between the four spectra is at intensity of 3000–3800 cm^{-1} representing hydroxyl (-OH) group stretching, and at intensity of 1600–1700 cm^{-1} representing carbonyl (–C=O) stretching. This may signify the involvement of hydroxyl groups in the binding of Ni, Cd and Cr. The presence of slight changes in those peak regions indicates the presence of biosorption. Interestingly, the peaks shifted with the presence of metal ions suggesting an interaction of metals with these functional groups.

In addition, the main functional groups responsible for a biosorption process are the hydroxyls, carbonyls, carboxyls, sulfonates, amides, imidazoles, phosphonates and phosphodiester groups as affirmed by [16] and [18]. Some of these groups are present on the *Trichoderma* sp. biomass and may interact with the metal ions. It is also reported that the binding of Ni(II) to the biopolymers occur mainly in the peptidoglycan and layer of the cell surface [14].

4. Conclusions

Trichoderma sp. have the ability to remove heavy metals in the order Cd > Cr > Ni. Dry *Trichoderma* sp. biomass functions well and efficient in removing metals ions than wet *Trichoderm*a sp biomass. There were differences in the positions of the peaks in FTIR graphs showing the shifting of all the peaks or bands influenced by the metal ions. A change in peak position in the spectrum of *Trichoderma* sp. after treating the sample contain either Ni, Cd, Cr and groundwater indicates that there are bindings of heavy metal onto the functional groups.

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