

Toxicity test of desorbed chromium from polluted soil around tannery industry in Yogyakarta-Indonesia by using *Brassica juncea* and *Helianthus annuus* L.

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Abstract. Indonesia's target to be a new industrial country makes a wide chance in the developing of many industrial sectors. The main problem is the disruption of environmental balance due to poorly managed liquid waste released by the industry. Heavy metal such as chromium (Cr) is the typical pollutant generated from tannery industry in Indonesia. Toxicity test of desorbed chromium ions from polluted soil around tannery industry in Yogyakarta-Indonesia by using *Brassica juncea* and *Helianthus annuus* L. has been carried out. In this research, soil samples were divided into four sample points based on the distances from the waste water point sources. Metal content and some physico-chemical properties including ash content, total organic carbon, and cation exchange capacity were determined in this study. Furthermore, the chromium ions adsorption and desorption from polluted soils were investigated and measured by atomic adsorption spectrophotometry (AAS). Thus, ecotoxicity tests of soil eluates from desorption experiments were performed with *Brassica juncea* and *Helianthus annuus* L. The results showed that sample III had the highest Cr content at 916 mg kg⁻¹. The adsorption isotherm of Cr ions into soils followed Langmuir isotherm with the highest maximum adsorption capacity value at 14.286 mg kg⁻¹ in the sample point IV (unpolluted soil). The optimum desorption of chromium occurred at pH 3. Toxicity test showed that Cr ions had toxic effect to *Brassica juncea* and *Helianthus annuus* L.

Keywords: Chromium, desorption, toxicity test, tannery industry.

INTRODUCTION

Tannery industry is an industry that treats a variety of raw leather, semi-finished leathers (pellicle and wet-blue leathers) to become finished leather. Apart from the positive impact, the industrial sector also has a negative impact. The leather tanning industry is categorized as one of the high polluting industries because of tanning activities that have an adverse effect on the environment. This is due to industrial wastes which if not managed properly. In fact, the government has implemented regulations and provisions regarding industrial obligations in processing waste, including those contained in Law of the Republic of Indonesia Number 32 year 2009 concerning Protection and Management of

Environment; also Indonesia Government Regulation Number 101 year 2014 regarding Hazardous and Toxic Waste Management.

Heavy metals are a serious threat today for living things and the environment. Heavy metals can be divided into two categories, namely essential and non-essential elements. All of the two categories have the potential to contain poisons (Nagajiyoti et al., 2010). As a dangerous chemical, heavy metals are substances that cannot be decomposed and remain behind for long periods of time in the soil. Leather tanning waste water include hazardous waste because they contain heavy metals such as chromium. The leather tanning process can only adsorb 60-80% chromium, which means that around 20-40% of

chromium will be carried in liquid waste and solid waste in the tanning industry (Mayasari and Sholeh, 2016). Thus, significant chromium concentrations are released into the environment through leather tanning industry (Sneddon, 2012).

Andaleeb et al. (2008) stated that the length of germination, roots and shoots decreased with increasing Cr content in the soil. Suherman et al. (2013) have also conducted research related to the toxicity test of Cu and Pb metals from the humus soil of the Harz Mountains (Germany) with *Lepidium sativum*. The properties of soil eluets slightly reduce the inhibitory effect of Cu on the growth of *Lepidium sativum*, especially at low concentrations compared to synthesis solutions. The success of germination depends on external factors. This implies that seeds must be supported by internal sensors capable of sending environmental signals into cellular mechanisms that lead to germination (Leymarie et al., 2009; Bailly et al., 2008; Kranner and Colville, 2011).

This study focuses on the impact of chromium contaminated soil and its effect on seed germination, especially for *Brassica juncea* and *Helianthus annuus* L. Studies of soil characteristics and adsorption are needed to determine relation between soil properties and adsorption capacity of contaminated soil. While the desorption study also needed to investigate the possibility of metal distribution in the soil. In addition, toxicity test of chromium needs to be done to evaluate the effect of heavy metals in plant growth.

EXPERIMENTAL SECTION

Materials

Soil samples were taken from three different sampling points (20-40 cm depth) at Piyungan tannery industry area of Yogyakarta-Indonesia. Sample point IV was appointed as unpolluted soil. The sample point I and II were taken at a location close (side area) to the industrial building, while sample point III just after the outlet of waste water point source. The materials used by pro-analysis qualities from Merck were barium chloride dihydrate ($\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$), magnesium sulfate heptahydrate ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$), standard solution of chromium 1,000 mg/L, iron (II) ammonium sulphate (FAS, $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$), potassium

dichromate ($\text{K}_2\text{Cr}_2\text{O}_7$), hydrofluoric acid (HF), sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), indicator of ferroin ($\text{C}_{36}\text{H}_{24}\text{FeN}_6\text{O}_4\text{S}$), tartaric acid, citric acid, oxalic acid, and Whatman 42 filter paper.

The apparatus in this research are analytical balance (Mettler Toledo AB54-S), digital balance sheet (ACIS AD 300i), shaker (Marius Instrumenten), and oven (Kirin). While instrumentation used are X-ray diffractometer (Shimadzu model XRD-6000), fourier transform infra-red (Hanna HI98103), hot plate-stirrer (Thermolyne Cimarec 1), a set of reflux device and an atomic absorption spectrophotometer (AAS, Perkin Elmer 3110).

Methods

Physico-chemical characterization of the soil samples

For the physico-chemical characterization of the soil samples, the processes were started with the drying step of the 10-15 g samples each at 105 °C for 24 h. The water content of soil samples was determined by the ISO 11465 (1993). Ash content of soil samples were determined by DIN CEN/TS 14775 (2004). Determination of organic carbon was done based on ASTM D 2974 (2000). The electrical conductivity is determined by DIN CEN/TS 15937 (2013). pH of soil samples was measured by the method of ISO 10390 (2005). While cation exchange capacity (CEC) is determined based on DIN ISO 11260 (1997). For functional groups of soil samples, the FTIR method was used for characterization. Data retrieval is repeated three times to calculate the standard deviation.

Metal determination

Determination of metal content based on the British Standards European Norm (BS EN) 13656 (2002) method. A total of 0.5 g of soil samples were extracted with the addition of 4 mL of 40% HF solution and shaken for 24 h. The mixture was added 12 mL of aqua regia and shaken back for 24 h. The filtrate is separated which is then measured by the metal content of the AAS instrument.

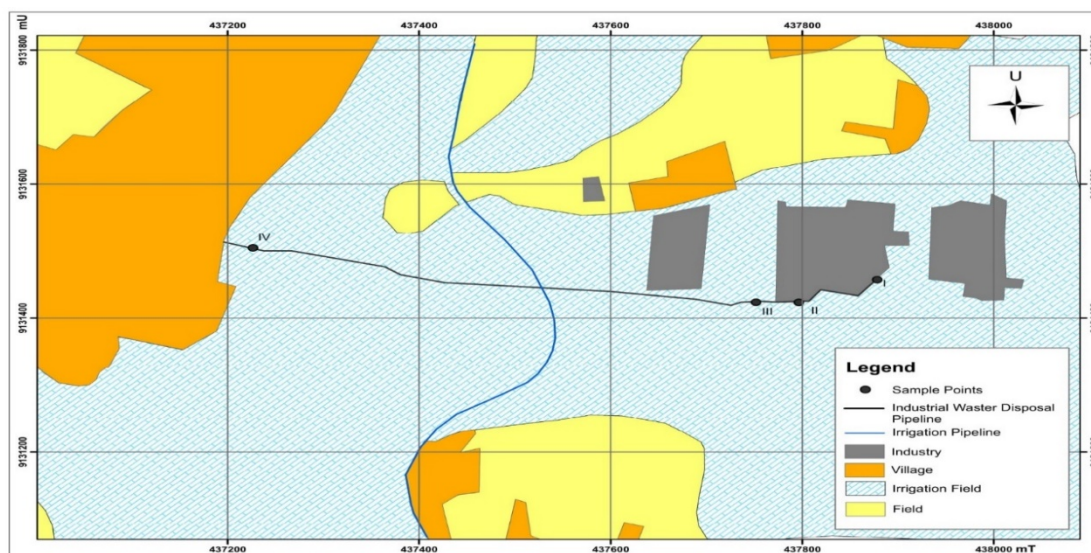


Figure 1. Soil sampling site at Piyungan Yogyakarta-Indonesia

Total Cr content determination

A soil sample with a mass of 2 g was put into a polyethylene bottle which was then added with 50 mL of aquabides. Furthermore, the pH of the solution was adjusted with variations in pH 3, 5, 7 and 9. The mixture was shaken for 24 h which was then centrifuged at a speed of 5000 rpm for 15 min. The sample was filtered with Whatman 42 filter paper and the filtrate was taken. Cr concentration in the filtrate after interaction was measured by the AAS instrument. Data retrieval is repeated three times to calculate the standard deviation. The study was carried out at $\pm 27^\circ\text{C}$ and a room pressure of ± 1 atm.

Chromium toxicity test with *Brassica juncea* and *Helianthus annuus* L.

Soil eluets filtered from desorption experiments (with variations in pH 3, 5, 7 and 9) and aquabides as blank were tested for toxicity with *Brassica juncea* and *Helianthus annuus* L. Cr synthetic solutions were prepared in pure water containing the same Cr concentration as desorption of soil eluates. As much as 5 mL of each solution (both eluates from desorption and synthetic solution) were pipetted into Whatman 42 filter paper which had been placed on the petridish. As a control, the same procedure is carried out with aquabides. A total of 25 seeds from *Brassica juncea* and *Helianthus annuus* L. are arranged on wet filter paper with a proportional distance from each other. Petridish plates were closed and incubated for germination at room temperature in darkness for 3 days. After 3 days, the root length and hypocotyl from the seeds were measured, then

the percent inhibition for each solution concentration was calculated.

RESULTS AND DISCUSSION

Soil samples used in this study were taken from the soil around tannery industry in Bantul, Yogyakarta. The soil samples studied were top soil with a depth of 0-30 cm. The results for the characterization of physicochemical properties of each soil sample are presented in **Table 1**.

Table 1 shows that the soil pH measured using H_2O extract solution has a higher value than KCl and CaCl_2 salt solutions. This is due to the release of cations when salt is added to the soil suspension. The release of cations will replace hydrogen ions in the soil, so hydrogen ions will be pushed into the soil solution which causes the concentration of the soil to approach the concentration of its natural conditions.

Ashraf et al. (2017) states that soil pH is an important factor that controls the geochemical ability of heavy metals in the solid phase and solution from the soil. Soil pH determines the adsorption and desorption processes and chemical speciation of Cr and other heavy metals in the soil. In addition, at high pH there will be deprotonation of the hydroxyl group of soil components which causes an increase in negative charge on soil binding sites so that metal cations can be adsorbed by soil through electrostatic interactions. Sample point III has the highest pH value and metal content and at sample point IV has the lowest pH value and metal content (as unpolluted soil). This evidence showed with the lower pH, metals will be

easier to leaching and cause thus distribute to a wider range in the soil.

Acidity of soil pH is influenced by the presence of active ions of hydrogen and aluminium ions in soil solutions. This comes from the dissociation of water in the form of OH^- and H^+ , as well as the release of Al^{3+} from the structure of clay minerals in the adsorption location on colloidal soil. The soil

pH value in **Table 1** is in the range of pH value 6.17-7.87 with the lowest pH value owned by sample point IV. The lower pH of sample IV is possibly due to the presence of H^+ released due to reaction of Al^{3+} and water molecules in the soil solution (Verheye, 2009).

Tabel 1. Physico-chemical properties and metal content of the soil samples around tannery Industry

Parameter	Sampling point I	Sampling point II	Sampling point III	Sampling point IV
pH				
a. H_2O	7.57	7.83	7.87	6.90
b. KCl	7.13	6.80	7.00	6.17
c. CaCl_2	7.33	7.07	7.13	6.67
Total organic carbon (mg C g^{-1} soil)	34.63	43.03	42.90	45.67
Soil organic matter (mg g^{-1} soil)	59.71	74.18	73.95	78.73
Cation exchange capacity ($\text{meq}/100 \text{ g}$)	0.80	1.23	1.04	1.32
Ash content (%)	17.62	17.10	20.69	15.23
Electrical conductivity ($\mu\text{S cm}^{-1}$)	1,329	1,978	2,533	211
Water content (%)				
a. Room temperature	8.49	10.45	16.16	9.98
b. 105°C	37.86	50.39	51.62	45.53
Cr total (mg kg^{-1})	459.4	706.8	916.2	167.1

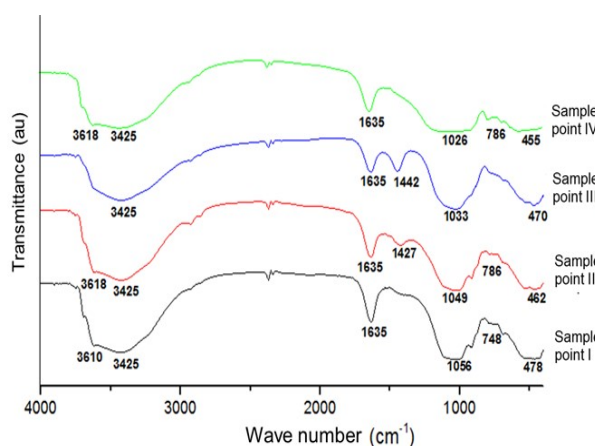


Figure 2. FTIR spectra's of soil samples

Soil organic matter consists of organic carbon (C-organic) as the main constituent component which is as much as 58% (de Brogniez et al., 2015). Soil organic matter has a functional group that can contribute negative charges to the soil. This negative charge is able to exchange cations in the soil so as to increase soil cation capacity. Cations that are bound in the soil by clays or organic matter can be replaced with other cations. The amount of cation is linearly related to the

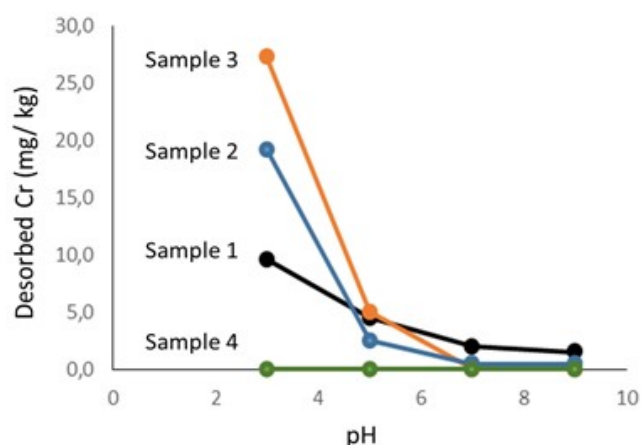


Figure 3. Effect of pH variation on the Cr desorption from the soil samples

amount of negative charge in the soil. The CEC results in **Table 1** showed that at sample point IV has the highest CEC value. These results indicate that the sample has the highest predominance of negative charge and is proportional to the number of cations that can be bound by the soil. This CEC value can also be correlated with the presence of organic C contained in sample point IV which shows the highest value as well. High levels of organic C indicate the increasing amount of soil

organic matter with the dominance of negative charges in the soil. As the results from Suherman et al. (2013), soil organic matter will be directly proportional to the value of soil cation exchange capacity (CEC) because soil organic matter will increase the negative charge in the soil with the results of sample point IV having the highest organic matter content and soil CEC.

Ash content shows the quantity of minerals and other inorganic ingredients in the soil. In **Table 1** it is shown that soil samples at sample point III have the highest ash content and sample point IV shows the lowest ash content. Determination of ash content is done by heating at high temperatures gradually to a temperature of 550 °C to oxidize all organic matter. This inorganic material which becomes ash can further be used to bind metal Cr in the soil when the levels of organic matter are low.

Electrical conductivity is the ability of the soil to conduct electric current. Electrical conductivity in the soil can be associated with the ions contained in it. Soil has the ability to conduct electricity through ions dissolved in the soil. Basically the electrical conductivity of the soil increases with increasing concentration of salts in solution. Electrical conductivity can also be related to the water content contained in it. Water in the soil can solvate ions which can then be exchanged with other ions. Based on **Table 1**, sample point III has the highest water content. The electrical conductivity values for sample points I, II and III are high because the average soil electrical conductivity is in the order of 10^3 (Rodríguez-Pérez et al., 2011; Suherman et al., 2013). At sample point IV it is in the order of 10^2 because at sample point IV is a sample point of unpolluted soil used as a comparison (with lower metal cationic existence).

The results of chromium analysis in **Table 1** showed that there is very high contamination in the soil around the flow of liquid waste from the tannery industry. Sampling point III shows soil samples with the highest Cr content and sampling point IV have the lowest Cr content. This is supported by ash content, electrical conductivity and the highest water content possessed by sample point III. The ash content in soil samples represents the content of inorganic material in the soil. At sample point III, the binding of Cr is carried out by the inorganic material it has. This is supported by the statement of Mandzhieva et al. (2014) that mineral mineral fraction is an important factor in the adsorption of metal cations when the levels of

organic matter in the soil are low. Inorganic materials that can play a dc role include Al, which is one of the dominant constituent elements of the soil. In its oxidation form, Al_2O_3 is amphoteric (Idrissova & Tumanova, 2014) so that at low pH Al_2O_3 is a base that is able to bind to metal cations.

Figure 2 shows negative groups that play a role in binding heavy metals in the soil sample. The four spectra show absorption at relatively equal wave numbers. Wave number of 3618 cm^{-1} , 3610 cm^{-1} and 3425 cm^{-1} show absorption of OH groups in carboxylic acids. The absorption band 1635 cm^{-1} shows the absorption of OH groups from silanol (Si-OH). The absorption band intensities in the area around 3618 cm^{-1} and 1635 cm^{-1} seems to be decreasing due to the reduction of OH groups on the soils after the adsorption process with metal ions. At sample points II and III which are sample points of contaminated soil there is absorption in the area of 1427 cm^{-1} and 1442 cm^{-1} which shows the vibration of bending C-H. Wave number 1056 cm^{-1} , 1049 cm^{-1} and 1033 cm^{-1} respectively at sample points I, II and III show absorption for stretching vibrations of Si-O and Si-O-Si. At sample point IV which is a sample of uncontaminated soil, there is a shift in the absorption band in the wave number area 1026 cm^{-1} . The peaks in the wave numbers of 478 cm^{-1} , 462 cm^{-1} , 470 cm^{-1} and 455 cm^{-1} for sample points I, II, III and IV respectively show Si-O-Si vibration, which indicates that the metal ion in binding to the surface of the soil not only through silanol groups, but also through siloxane groups (Si-O-Si).

Figure 3 revealed the phenomenon of declining chromium desorption as pH increases. The high concentration of chromium desorbed in an acidic condition caused by speciation of Cr in the soil eluates due to the influence of pH. This is supported by a statement from Dias-Ferreira et al. (2015) that desorption of chromium from soil into the solution is most significant at lower pH. The relatively low concentration of chromium desorbed from highly polluted soil samples ($< 100\text{ mg/kg}$) just in comparable number to the previous results of Zn desorption from illegal land fill area in Yogyakarta (Amaliyah et al., 2019).

Toxicity tests of desorbed chromium by using *Brassica juncea* and *Helianthus annuus* L.

The toxicity test of chromium against *Brassica juncea* and *Helianthus annuus* L. were studied by germination using eluates solution containing heavy

metal from the results of desorption variation of pH of the solution. Although the level of heavy metals in the soil around the tannery industry exceeds the permissible threshold (Table 1), the fraction of metal which is desorbed in soil eluates using a variation of pH is quite low, as shown in **Figure 3**. In the toxicity tests with *Brassica juncea* and *Helianthus annuus L.*, growth of root and hypocotyl were influenced by the presence of Cr metal in eluates from the desorption experiments.

In the toxicity test with *Brassica juncea* (**Figure 4**), growth of root and hypocotyl were influenced by the presence of Cr solution from the results of desorption experiments. From the toxicity test, there is a direct relationship between the severities of *Brassica juncea*'s growth response with increasing metal concentration. However, at the lowest concentration of Cr eluates (**Figure 4a**, unpolluted soil), the eluates support the growth of the root and hypocotyl instead of inhibited the elongation. This phenomena possibly due to the eluates containing the nutrients extracted from the

soil samples that stimulate the seedlings growth. In addition, Cr solution in both eluates and synthetic clearly inhibited the growth of the *Brassica juncea*. But, the hypocotyl growth responded more sensitive toward Cr than root growth.

The effect of Cr eluates and synthetic solution obviously inhibited the growth of *Helianthus Annuus L.* seedlings as can be seen in **Figure 5**. At the lowest of Cr eluates (**Figure 5a**), the solution caused a positive effect on the seedling growth. This condition just in accordance to the Cr eluates effect on the *Brassica juncea* (**Figure 4a**), that the existence of soil nutrients at the eluates could support germination process. In case of the synthetic solution without DOM (dissolved organic matter) and soil nutrients, the growths (root and hypocotyl) were adversely affected by the presence of chromium solution. And there was strong relationship between the severity of the plant responses and the increasing of Cr concentration in the synthetic solution.

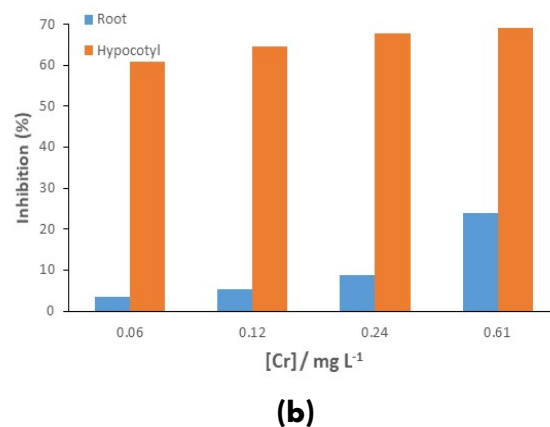
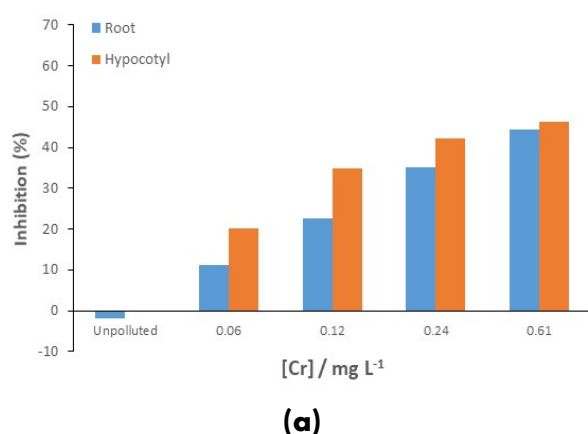


Figure 4. Toxicity test of Cr eluates (a) and Cr synthetic solution to the *Brassica juncea* (at pH neutral)

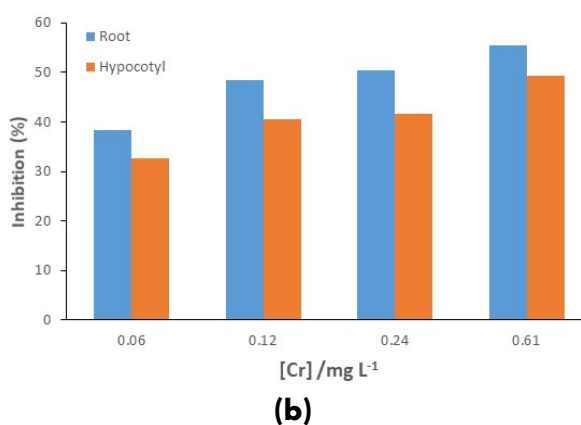
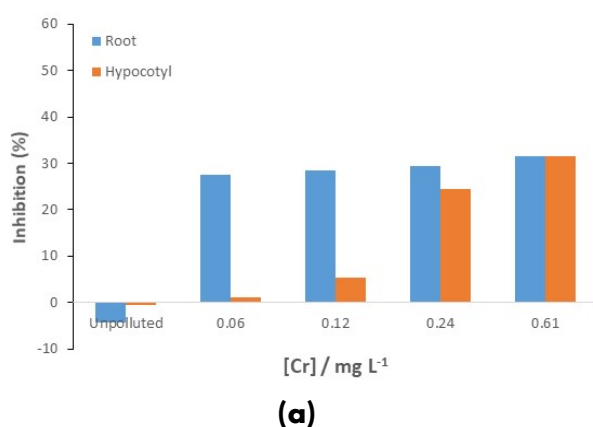


Figure 5. Toxicity test of Cr eluates (a) and Cr synthetic solution to the *Helianthus Annuus L.* (at pH neutral)

CONCLUSIONS

The physicochemical properties of soil samples have a relationship with the profile of chromium concentration, with the condition that all soil sampling sites are highly polluted with the chromium in comparison to the chromium concentration from the unpolluted soil samples (sampling site IV). In the desorption study, it was revealed that chromium was desorbed from the soil structure at the optimum pH 3. Toxicity test shows that Cr has a toxic effect on *Brassica juncea* and *Helianthus annuus* L. Growth (roots and hypocotyl) in plants decreases with the increasing chromium concentration in eluates and synthesis solution.

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