BIODIVERSITAS
Volume 17, Number 1, April 2016
Pages: 84-89

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d170112

Mitigation of mercury contamination through the acceleration of vegetation succession

WIWIK EKYASTUTI[1,♥], ENY FARIDAH[2], SUMARDI[2], YADI SETIADI[3]
[1]Faculty of Forestry, Tanjungpura University. Jl. Imam Bonjol, Kotak Pos 6271, Pontianak 78124, West Kalimantan, Indonesia. Tel.: +62-561-767673, 764153, Fax.: +62-561-764153, ♥email: wieky_serdam@yahoo.co.id
[2]Faculty of Forestry, Gadjah Mada University. Jl. Agro Bulaksumur Yogyakarta 55281, Indonesia
[3]Faculty of Forestry, Bogor Agricultural University. Jl. Lingkar Akademik Darmaga Bogor 16680, West Java, Indonesia

Manuscript received: 8 January 2016. Revision accepted: 10 February 2016.

Abstract. *Ekyastuti W, Faridah E, Sumardi, Setiadi Y. 2016. Mitigation of mercury contamination through the acceleration of vegetation succession. Biodiversitas 17: 84-89.* The success of the restoration of the tailings ex-gold mining through the succession is highly dependent on the ability of plants to grow and adapt to the troubled land. Restoration through natural succession takes a very long time. Therefore, human intervention is required to accelerate the succession. The purpose of this research was to improve the effectiveness of mitigation of mercury contamination through the acceleration of vegetation succession. This research has been carried out in a greenhouse using an experiment with a completely randomized design. There are 8 treatment consists of four indigenous species (*Dillenia excelsa, Melastoma affine, Cinnamomum porrectum* and *Casuarina junghuhniana*) grown alone (one species) and collective (more than one species) in the tailing media with a mercury content of 20 ppm. The results showed that the planting collectively have a mutually supportive interaction, so that increased the plant growth. In addition, collective planting two or four different species of plants, and the *D. excelsa* itself could decrease the concentration of mercury in the tailing. The acceleration of vegetation succession through the right choice of plants species and planting collectively, capable to increasing the potential of mitigation of mercury contamination in the tailings.

Keywords: Ex-gold mining succession, mitigation of mercury contamination, tailing

INTRODUCTION

Rehabilitation of the tailing areas of ex community gold mining in West Kalimantan, have been urged to do. One phase of the rehabilitation activities is revegetation. Through revegetation activities are expected to not only lower the mercury contamination in the tailings, but also improve the local micro-climate conditions. Revegetation can be done artificially by humans and naturally through the process of succession. The success of the improvement of the tailings of ex community gold mining through a succession are highly dependent on the ability of plants to grow and adapt to the troubled land, so the use of restoration techniques in revegetation is one right choice (Ekyastuti and Roslinda 2015). Restoration is an effort to repair or restore the condition of the damaged area by forming the structure and function close to the original condition (RECA Project 2014). Restoration through natural succession has been proven to reduce the levels of mercury contamination in the tailings, but the process takes a very long time. Previous research has found that the succession of the tailing areas of ex gold mining running very slow. In the tailing areas of ex community gold mining that have been abandoned for five years is still dominated by groundstorey and shrubs (Ekyastuti and Roslinda 2015). Therefore, human intervention is required to accelerate the succession. Human intervention can be done through increasing revegetation activities deliberately in the tailing areas of ex gold mining.

The use of indigenous species is preferred in re-vegetation activities which refers to the restoration techniques. This meant that the goal to forming the structure and function close to the original condition can be achieved. In addition, the use of indigenous species can also maximize the success of replanting, due to the suitability of species where they grow. Secondary forests around the tailing areas of ex community gold mining in West Kalimantan has excellent potential in providing a source of indigenous species for revegetation. Ekyastuti and Roslinda (2015) research in the tailing areas of ex gold mining found 10 indigenous species in Mandor location and 18 indigenous species in Menjalin location. This indicates that the use of indigenous species for revegetation is feasible in both locations. Potential and equal opportunities is also happening in mined land in other places.

Considering the succession in the first five years of the tailing areas are still dominated by groundstorey and shrubs, as previously described, it is necessary to know the role of species of these plants to improve revegetation success. Population groundstorey and shrubs are also thought to have a role in reducing mercury (or other pollutants) on the ground. However, there is no information that conveys the formulation or planting utilizing this groundstorey and shrubs. Therefore, this study aims to improve the effectiveness of mitigation of mercury contamination through the acceleration of vegetation succession. Acceleration of the vegetation succession is done by using some indigenous species elected.

MATERIALS AND METHODS

Research has been carried out in a greenhouse using an experiment with a completely randomized design. As an experimental material were four indigenous species elected refers Ekyastuti et al. (2016), which consists of two species of shrubs that was *Dillenia excelsa* and *Melastoma affine* and two species of woody plant that was *Cinnamomum porrectum* and *Casuarina junghuniana* (Figure 1). Selection of species was based on the ease of the species found in the study site and easy too propagation. Furthermore, the treatment consists of eight levels (planting with species grown alone (one species) and collective (more than one species)), and each treatment was repeated five times. As a plant growth media were tailings of ex gold mining + mature compost 1:1 (v:v) (Ekamawanti and Ekyastuti 2010), with a mercury content 20 ppm. It refers to Ekyastuti et al. (2016) that with the mercury content 20 ppm, these four species of plants still can grow well. Mercury added to the media using techniques of Rabie (2005).

Analysis of variance is done using statistical software program SAS 13 for data growth of plants that collected during the study, namely: (a) the increase of height (cm), (b) the increase of diameter (mm), (c) increase the number of leaves, (d) the ratio of roots and shoots, and (e) the total dry weight of the plant. At the end of the study was also carried out analysis of total mercury content in the roots, shoots and media (ppm) in the Laboratory of Baristand Pontianak using standard SNI 06-6992.2 2004. The data will be used as baseline data to calculate: bioconcentration factor, translocation factor and tolerance index following the technique of Rabie (2005).

RESULTS AND DISCUSSION

Results

Plant growth as a manifestation of the advancement of succession

In general, the growth of four indigenous species are in a good condition and healthy. Found as many as 10% of the crop showed symptoms of mercury poisoning but only until early symptoms of poisoning that are leaves turn yellowing in the bud and leaf edge become browning (Figure 2), and plants still can grow normally until the end of the study. Indigenous species that show symptoms of mercury poisoning are three individual of *D. excelsa* and three individual of *C. porrectum*. The results showed that at all levels of treatment, except in the number of leaves D. excelsa which planted individually, there were no differences in plant growth (Table 1).

The content of mercury in the media and plant tissue

Six months after planting, the results of the analysis of mercury in the tailings media vary considerably (Table 2). The decrease of mercury content in the media are from 20 ppm to 1.46 ppm until <0.002 ppm. Another fact that derived from this data is the discovery of a tendency that the collective planting (more than two species) and planting *D. excelsa* singly, faster to decrease of mercury content in the media than other treatments. The controls are the same medium but without the plant, that is used as a comparison to see the effect of plants in reducing mercury in the media. Mercury concentrations in control media showed the highest value (1.46 ppm), but not much different from the media of *C. junghuniana* that planted singly (1.44 ppm).

Mercury analyzes were also performed on plant tissue at the end of the study (Table 3). At all indigenous species, a different treatment does not cause the differences in mercury concentrations in the plant tissues. However, there is a tendency that *C. junghuhniana* and *D. excelsa* has a higher ability to accumulate mercury, compared with two other species (Figure 3). Meanwhile, for the ANOVA among the treatments (that is the sum of all the mercury content of the plants in each treatment) was significantly different. In order to track the differences among the treatments, we carried out further testing using DMRT (Table 4).

Based on the results of DMRT can be explained that the mercury content in plant tissue on collective planting of four species found the highest, followed by the collective planting of two species of woody plant, then the collective planting of two species of shrubs and planting single of *C. junghuhniana* and *D. excelsa*. Between *M. affine* and *C. porrectum* that grown singly did not show differences in mercury content in the plant tissues, and have the lowest content of mercury in the plant tissues.

Bioconcentration factor, translocation factor and tolerance index

Bioconcentration factor, translocation factor and tolerance index are measured to determine the distribution of mercury in the media and in the plant, as well as the ability of plants to face the presence of mercury contamination (Table 5). Bioconcentration factors are measured to determine where mercury accumulated, in plant tissue or in the media. The result showed that the majority of plants, except *C. junghuhniana* and *C. porrectum* that planted alone, have a value of bioconcentration factor > 1. This means that the accumulation of mercury generally occurs in the plant tissue, not in the media (Rabie 2005). Based on this data, *C. junghuhniana* and *C. porrectum* (woody plant) does not have the ability of mercury accumulation in plant tissues when planted alone. However, if they are planted collectively with other species, these plants have the ability of mercury accumulation in plant tissue. This suggests that the relationships formed among species of these plants is a co-founding or support each other to absorb mercury. The same trend also occurred in the group of herbaceous plants (*D. excelsa* and *M. affine*).

Figure 1. Indigenous species were planted. A. *D. excelsa*, B. *M. affine*, C. *C. porrectum*, D. *C. junghuniana*, and E. Experimental planting in the greenhouse

Figure 2.A-B. Early symptoms of mercury poisoning that appears on *D. excelsa*

Table 1. Results of the analysis of a varian (Anova) of plant growth

Variable of growth	Indigenous species	Average	P-value	Anova
The increase of height (cm)	*D. excelca*	2.00	0.178	n.s.
	M. affine	1.09	0.369	n.s.
	C. porrectum	1.11	0.362	n.s.
	C. junghuniana	0.25	0.786	n.s.
The increase of diameter (mm)	*D. excelca*	0.40	0.679	n.s.
	M. affine	2.67	0.110	n.s.
	C. porrectum	0.75	0.493	n.s.
	C. junghuniana	2.00	0.178	n.s.
Increase the number of leaves	*D. excelca*	0.93	0.420	n.s.
	M. affine	2.00	0.178	n.s.
	C. porrectum	4.96	0.027	*) s
	C. junghuniana	0.63	0.548	n.s.
The total dry weight of the plant (g)	*D. excelca*	2.66	0.110	n.s.
	M. affine	0.61	0.560	n.s.
	C. porrectum	2.11	0.164	n.s.
	C. junghuniana	0.99	0.399	n.s.
The ratio of roots and shoots	*D. excelca*	2.06	0.170	n.s.
	M. affine	0.79	0.476	n.s.
	C. porrectum	0.55	0.593	n.s.
	C. junghuniana	0.26	0.776	n.s.

Note: ns = non significant; *) s = significant

Table 2. Concentration of total mercury in the media six months after treatment

Treatment	Concentration of total mercury in the media (ppm)
Control	1.46
D. excelca (De)	< 0.002
M. affine (Ma)	0.278
C. porrectum (Cp)	0.758
C. junghuniana (Cj)	1.44
De + Ma	< 0.002
Cp + Cj	< 0.002
De + Ma + Cp + Cj	< 0.002

Table 3. ANOVA of mercury content in plant tissue

Indigenous species	Average of mercury (ppm)	P-value	Anova
Among plants:			
D. excelca	1.89	0.295	n.s.
M. affine	0.40	0.400	n.s.
C. porrectum	0.40	0.400	n.s.
C. junghuniana	2.85	0.203	n.s.
Among the treatments (7 planting)	3.56	0.0001	**) v.s.

Note: ns = non significant; **) v.s = very significant

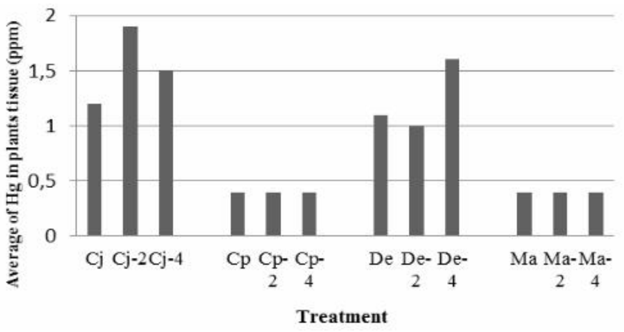

Figure 3. The average of mercury content in plant tissue (ppm)
Note: Cj = *C. junghuhniana*, Cp = *C. porrectum*, De = *D. excelca*, Ma = *M. affine*; 2 = collectively planted two different species; 4 = collectively planted four different species

Table 4. DMRT of mercury content in plant tissue

Treatment	Average of mercury content (ppm)	DMRT
De + Ma + Cp + Cj	3.85	a
Cp + Cj	2.30	b
De + Ma	1.35	c
C. Junghuhniana (Cj)	1.20	cd
D. excelca (De)	1.10	cd
M. affine (Ma)	0.40	e
C. Porrectum (Cp)	0.40	e

Description: Mean followed by the same letter on DMRT showed no significantly different results

Table 5. Bioconcentration factor, translocation factor and tolerance index

Indigenous species	Bioconcen-tration Factor	Trans-location Factor	Tolerance Indexs (%)
D. excelca	578.95	0.22	148.79
M. affine	1.44	1.00	133.56
C. porrectum	0.53	1.00	97.06
C. junghuniana	0.83	0.17	113.04
D. excelca-2	526.32	0.27	119.74
M. affine-2	210.53	1.00	145.83
C. porrectum-2	210.53	1.00	65.52
C. junghuniana-2	1000.00	0.12	183.33
D. excelca-4	842.11	0.15	216.79
M. affine-4	210.53	1.00	180.00
C. porrectum-4	210.53	1.00	164.29
C. junghuniana-4	789.47	0.15	171.43

Description: -2 = collectively planted two different indigenous species and -4 = collectively planted four different indigenous species

The value of translocation factor is measured to determine in what part the mercury accumulated in plants, whether in the roots or shoots. If the value of the translocation factor ≥ 1 means that mercury accumulated in the shoots, whereas when the value < 1 means that the mercury accumulated in the roots (Rabie 2005). The value of factor translocation of four indigenous species (Table 5) showed that *C. junghuhniana* and *D. excelsa* accumulate mercury in the roots, while *C. porrectum* and *M. affine* accumulate mercury in the shoots. This phenomenon does not change in plants grown singly (alone) and collectively.

The value of tolerance index is used to see the level of tolerance of plants to mercury. If the value of the tolerance index <30% = low (intolerant), 31-70% = moderate tolerance and > 71% = high tolerance (tolerant). From Table 5 it is known that except the *C. porrectum* that grow together with *C. junghuhniana*, three other indigenous species have tolerance index 97.06% - 216.79%. This means that almost all species of plants that used in this study have a high tolerance for mercury. Another phenomenon is detected that the collective planting four indigenous species together always show the highest level of tolerance than the other treatments.

Discussion

The growth of the plant as a manifestation of success in addressing the problem of mercury

The success or failure of plants to grow normally, describes the ability or the failure of plants to exploiting the potential of media in order to supporting and overcoming the limitations. The areas of ex gold mining (tailings) have many limitations as a medium for plant growth. This media belongs to the criteria of degraded land because the nutrient content is very low, there is no organic matter, acidic to very acidic pH, CEC is very low, and contains of mercury (Ekamawanti and Ekyastuti 2010). Furthermore, it also made clear that these limitations can be minimized by the addition of mature compost in the ratio 1: 1 (v:v). Therefore, in this study we used tailings + mature compost as a plant growth media with a ratio 1: 1 (v:v). The result is a better fertility rate, making it feasible to be used as a medium to plant growth. This is evidenced from the plant growth response that normal.

At the end of this study, the concentration of mercury in the media become much lower ranged between <0.002 ppm to 1.46 ppm. The decrease is due to several reasons, including: absorbed and accumulated by plants or evaporates into the atmosphere due to volatilization (Wang 2004). Mercury uptake by plants can occur through a process of phytoextraction or phytostabilization (Sarma 2011). Decreasing the concentration of mercury in the soil is an indicator of the occurrence of metal binding process by roots exudates. In many cases, soil microorganisms have a very important role in assisting this process of decline of heavy metals in the soil (Prasetyawati 2009). Low concentrations of mercury in the media will be followed by the successful plant growth. This condition proves that the four indigenous species used in this study has the ability to accumulation of mercury. This statement is supported by the bioconcentration factor in most of the plants in all treatments > 1, which means the mercury accumulated in the plant tissue.

Vegetative growth as the response of plants to the site conditions showed that most plants can grow well and healthy. Although not differ in terms of growth and total mercury concentrations in plant tissue, there is a tendency that the planting collectively have a better plant growth if compared with the planting singly. The same trend is also seen in its ability to accumulate mercury in plant tissue. This is thought to be caused by the rapid reduction of mercury in large amounts, because absorption is done jointly with another individual. So the opportunity to grow better is higher. This statement is supported by the results of the analysis of mercury in the media. Planting collectively is causing the mercury content in the media to be much lower at <0.002 ppm, while the planting singly is 0.278 to 1.46 ppm. Except *D. excelsa* that planted singly, the mercury content is <0.002 ppm. This indicates that specific to *D. excelsa*, not only planted collectively but also planted singly capable to lowering the concentration of mercury in the media <0.002 ppm. Safety threshold of mercury in the soil according to the Decree of the Minister of Environment No. 202 of 2004 is 0.005 ppm.

Based on the value of the translocation factor, it is known that in *C. junghuhniana* and *D. excelsa* accumulate mercury in the roots, while *C. porrectum* and *M. affine* accumulate mercury in the shoots. Thus, the process of remediation of mercury by *C. junghuhniana* and *D. excelsa* is phytostabilization, while *C. porrectum* and *M. affine* is phytoextraction (Fulekar et al. 2009; Sarma 2011).

In most plants are tolerant of heavy metals, the remediation process is done through phytostabilization. This is because the process phytostabilization cause heavy metals (mercury) substituted into the tissues of plants and will soon be accumulated in a safe place. A safe place is generally at the root vacuoles. Thus, the process of photosynthesis is not inhibited because mercury does not enter into the leaf tissue. Conversely, if the remediation of mercury through phytoextraction process, the mercury will be up to the leaf tissue so that the process of photosynthesis to be blocked. This disorder occurs through the inhibition of the light reaction and the dark reaction, caused by the substitution of the central atom of chlorophyll and magnesium by mercury (Patra and Sharma 2000; Wang 2004). Therefore, the plants which remediate mercury through phytoextraction process tend to be lower tolerance to mercury. This causes the visually growth *C. junghuhniana* and *D. excelsa* much better than *C. porrectum* and *M. affine*.

Opportunity of increase in mitigation of mercury contamination through the acceleration of vegetation succession

Mitigation of mercury contamination biologically using a plant is called phytoremediation. Phytoremediation is a techniques of mercury reduction which the most inexpensive and safe for the environment (Sarma 2011; Project RECA 2014). Many researches have been carried out on phytoremediation. As a result, today it has been reported there are about 500 species of plants from 101 families, which have the ability to remediate heavy metals (Sarma 2011). However, only a few were specifically reported to remediate mercury. The ability of the plant as a phytoremediator is specific to the kind of heavy metal

(metal-specific) (Ward and Singh 2004; Palapa 2009; Sarma 2011). Therefore, plant species are able to remediate lead may not be able to remediate mercury as well.

C. junghuhniana and *C. porrectum* (woody plant) and *D.excelsa* and *M. affine* (shrubs) are four indigenous species that used in this study. They have the ability as phytoremediator mercury. This study can prove that the four indigenous species are able to grow well in the tailing media containing mercury 20 ppm. In addition, the four indigenous species are also able to accumulate mercury in plant tissue with diverse abilities and different places (plant tissue). When compared with the planting singly, planting collectively have a tendency to grow better and decrease the concentration of mercury in the media much faster (<0.002 ppm). This is evident from the results of the analysis of mercury content in the plant tissue. Planting collectively four indigenous species (3.85 ppm) and two indigenous species of woody plant (2.30 ppm), significantly has a higher ability to absorb mercury compared to collective planting two species of shrubs (1.35 ppm) and planting singly (0.4 to 1.2 ppm). However based on the value of tolerance index, all these plants are tolerant of mercury with the category of moderate to high. This proves that the four species of these plants are phytoremediator mercury (as previous explanation) and can be used to remediate mercury from the soil.

Four indigenous species used in this study is a local species, obtained from the remaining forest in the Nature Reserve in Mandor (West Kalimantan), a place where the community gold mining is taking place. The natural seedlings of the four species of plants are found in abundance in this area. This indicates that the remaining forest surrounding the community gold mining have a good potential as a source of seed.

Naturally, in the tailing areas of ex community gold mining will be occurred the succession. However, from a previous study showed that the succession process in the tailing ex community gold mining run very slowly. The succession after lasting more than five years is still dominated by groundstorey and shrubs (Ekyastuti and Roslinda 2015). So to speed up the repairs required active action of people who deliberately make the succession process run faster (acceleration). Based on the explanations that have been presented previously, it can be concluded that the chances are very large to increase the mitigation of mercury contamination through the acceleration of the vegetation succession. At gold mining areas in Mandor, West Kalimantan, the acceleration by utilizing *D. excelca*, *M. affine*, *C. porrectum*, and *C. junghuhniana* which planted collectively are highly recommended for revegetation purposes simultaneously mitigate mercury contamination in the tailings. However, this research is still a basic research. Further fieldwork studies are to be continued on stable surfaces established on waste stockpiles where particular species combinations would be planted.

From this study obtained results that: (i) the planting of four indigenous species namely *D. excelca*, *M. affine, C. porrectum,* and *C. junghuhniana* collectively cause interactions mutually support each other so that mutual benefit, (ii) planting collectively of two or four species plants, and planting *D. excelsa* singly capable to lowering the concentration of mercury in the media until <0.002 ppm, and (iii) plant tissues where the accumulation of mercury in *C. junghuhniana* and *D. excelsa* are in the roots (phytostabilization), whereas in *C. porrectum* and *M. affine* are in the shoots (phytoextraction). Based on the summary of the results can be concluded that the opportunities for improving mitigation of mercury contamination through vegetation succession acceleration is very great.

ACKNOWLEDGEMENTS

Our thanks go to the Directorate General of Higher Education (DIKTI), Republic of Indonesia on the funding for this research through research scheme Disertation Doctor.

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