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Research Article

The potential of wild vegetation species of *Eleusine indica* L., and *Sonchus arvensis* L. for phytoremediation of Cd-contaminated soil

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Abstract: Phytoremediation has been intensively studied due its costs effectiveness and environmentally sound. Studies of heavy metal pollution phytoremediation has been done in develop countries, but still limited in Indonesia. This study aims to explore the potential of wild plant species *Eleusine indica* L. and *Sonchus arvensis* L. as an agent of phytoremediation on Cd-contaminated soil. This study was done descriptively in Pujon, Malang, Indonesia, to test the ability of two species of wild plants *E. indica* and *S. arvensis* in absorbing Cd. Along this research, plant growth and the concentration of Cd in roots, stems and leaves, was monitored. Plant growth was measured every week for three months. The plant roots, stems, and leaves collected separately, then analyzed its Cd levels. The results showed that both of two species of wild plants grew well on soil contaminated Cd. Plant roots can accumulate higher Cd than the stem part. In addition, *E indica* has the ability to accumulate Cd higher than *S. arvensis*, i.e. 57.11% and 35.84%, respectively.

***Keywords**: cadmium, E. indica, phytoremediation, S. arvensis*

Introduction

The relatively high pollution intensity of farmlands in Java is commonly triggered by the intensive use of agrochemicals (fertilizers and pesticides). In 2002, intensive agricultural land areas in Java, was reported have been contaminated with Pb, Cd, Cu, and Zn from fertilizer and pesticides. For example, in the regions of Brebes and Tegal, the content of Pb in soil has crossed the threshold value (12.75 mg/kg). Besides, Hamzah et al. (2016) reported that the content of the Cd detected in Batu was 2.26 mg/kg. Pujon sub district as a horticultural production center in Malang also needs attention, because of high input of agrochemicals fertilizer.

The intensity of land use and high input of agrochemicals fertilizer will trigger a high accumulation of heavy metals, including Cd. This problem should be solved as soon as possible because it can interfere the soil and plant health as well as the human body. The high content of heavy metals in soil will influence biological processes in the soil. The biological process refers to the activity of soil organism which has an important role on decomposition of organic matter and soil productivity. The next impact was the disturbance of provisioning of N, P, S and C and other elements. It will hinder plant growth as well as the declining quality of plant production.

These problems can be overcome with the technology of phytoremediation because it is considered as the less expensive technology. The use of weed plants for the phytoremediation is getting attention at this time, because this technology is simple and cheap. Phytoremediation technology is the cheapest technology to remediate contaminated soil, compared to other technologies (McMohan, 2000; Moosavi and Mohamd, 2013). Phytoremediation of heavy metal contaminated soils is an emerging technology that uses plants to extracts or inactivates metals in soils (Macek et al., 2000). It is defined as the engineered use of green plants (including grasses, shrubs and woody species) to remove, concentrate, or render harmless such environmental contaminants as heavy metals, trace elements, organic compounds, and radioactive compounds in soil or water (Hinchman et al., 1996). It is environmentally friendly, of low cost, in situ applicable technique for the clean up of sites contaminated with toxic metals or organic pollutants.

The advantages of this technology compared with other remediation technology is the capability of the rizhospere to absorb and prevent the release of pollutants (Aremu et al., 1995). Plants show several response patterns to the presence of potentially toxic heavy metal ions. Most are sensitive even at low concentrations, others have developed resistance and a reduced number of them behave as hyper accumulators of these toxic metals (Schat et al., 1999), and reduce a large amount of contaminants (Sabeen et al., 2013). A phytoremediation competent is a plant species that produces high shoot biomass and accumulates the pollutant or its metabolites in the above-ground part, without any adverse effects on plant growth Some other researchers have already utilize certain plant species for the purposes of phytoremediation, such as *Ipomoea aquatica* (Bhaduri and Fulekar, 2012) and *Medicago sativa* (Wang et al., 2012) and some other plant species.

This research was aimed to explore the potential of two wild plant species that dominantly grow around agricultural lands as agents of phytoremediation of Cd contaminated soils.

Materials and Methods

This research was conducted descriptively in the field in Pujon sub district of Malang, Indonesia. Before planting, the soil was analyzed to determine pH (H_2O), the C-organic (Walkley and Black), N (Kjeldahl), (Walkley & Black), N-total (Kjedahl), P-total (Olsen), K-total, CEC (Ammonium Acetate pH 7.0). Cd content was analyzed using AAS (Atomic Absorption Spectrometry). Soil samples were taken randomly at several points of production lands of onions and vegetables such as carrots and potatoes. Composites soil samples were taken at a depth of 10 cm. A descriptive experiment was conducted to test the potential of *Eleusine indica*, L, and *Sonchus arvensis* L. for remediation Cd contaminated soils. Both species are dominant wild plants that grow around the farmland of Pujon sadistic. The two plant species were planted in the prepared plots. Observation of the plant growth was done every week until the plant was three months old. The parameters observed were plant growth and concentration of Cd in plant roots, stems and leaves. At harvest, the plant roots, stems, and leaves were collected separately. Plant samples were then washed with distilled water to remove soil and put into oven at a temperature of 6o^{0}C for 72 hours. Dried plant samples were then analyzed to determine the Cd content using AAS (Atomic Absorption Spectrophotometer). The total concentration of Cd in soil and plant samples were analyzed according to the methods developed by AOAC (1990). Dry soil and plant samples (1.00 g for each sample) was added to the digestive tube with 1 mL of concentrated nitric acid (HNO_3) and 5 mL of 70% chloric acid ($HClO_4$) and left overnight. Then the sample was heated at 100 ° C for 1 hour 30 minutes and after increased to 130°C for 1 hour. The temperature for the digestion of both was increased to 150°C for 2 hours 30 minutes (or until all the yellow steam is exhausted). After all the yellow steam exhausted, the temperature was then raised again to 170 ° C for 1 hour. The final temperature for the digestion of the sample was 200°C for 1 hour (steam white formed).

Sample digestion was complete when a white precipitate was formed and 1 mL of a clear solution. After digestion, the sample was filled with distilled water up to the 10 mL and then filtered through a MM 640 W Whatman filter paper. Analysis for the total concentration of Cd from each extract was done with AAS with various Cd standard solutions as a comparison.

Results and Discussion

Soil characteristics and Cd contamination

The initial soil chemical properties indicated that the soil had the following characteristics: pH (6.18), C-organic (1.86%), N (0.11%), P (0.64%), K (0.09%), and CEC (20.29 meq/100g). The low of C-organic content and CEC value indicated that soil fertility in the area was also low. Soil pH is usually regarded as the most significant variable influencing Cd uptake from the soil (Grant and Sheppard, 2008). Soil organic matter influences Cd bioavailability. Soils with higher organic matter have higher cation exchange capacity, which increases Cd adsorption. Soil pH and organic matter may interact in their effects on Cd availability. Organic matter has been found to reduce Cd concentration in the soil solution in contaminated soils at pH below 6.0 and increase soil concentration at pH 6.0-8.0. It was proved by the results of Cd content on the soil, which is exceeded a threshold value (2.39 mg/kg).

The high content of Cd was obtained supposedly derived from fertilizer and pesticide residues that have accumulated in the long term. P fertilizers are considered a major anthropogenic source of soil pollution with Cd (Bogdanovic et al., 1999). Cadmium occurs naturally in phosphate rock. All of the Cd in the rock phosphate is transferred to the single super phosphate (SSP) or triple super phosphate (TSP). Depending on the rock phosphate source, SSP can contain from 2 to more the 40 mg/kg and TSP can have from less than 10 to over 100 mg/kg Cd content (Van Kauwenbergh, 2001). Therefore, accumulation of phosphate used in the long term will lead to the accumulation of Cd element, especially if input is less than crop removal.

Grant (2011) showed numerous studies showing the application of P fertilizers containing 20 to 50 mg/kg of Cd led to significant increases in the Cd concentration of the soil. The normal amount of cadmium in the soil is below 1 mg/kg (the average content of Cd in soils is 0.4 mg/kg), but the Cd content in the soils studied was 2.39 mg/kg. This indicates that the content of Cd in the soil studied was above a threshold value. The heavy metal of Cd is more easily absorbed by plants compared to other heavy metals such as lead. Cd, contrary to the other heavy metals, is soluble in water, exhibiting high mobility in soil, thus the soil and crops become easily contaminated.

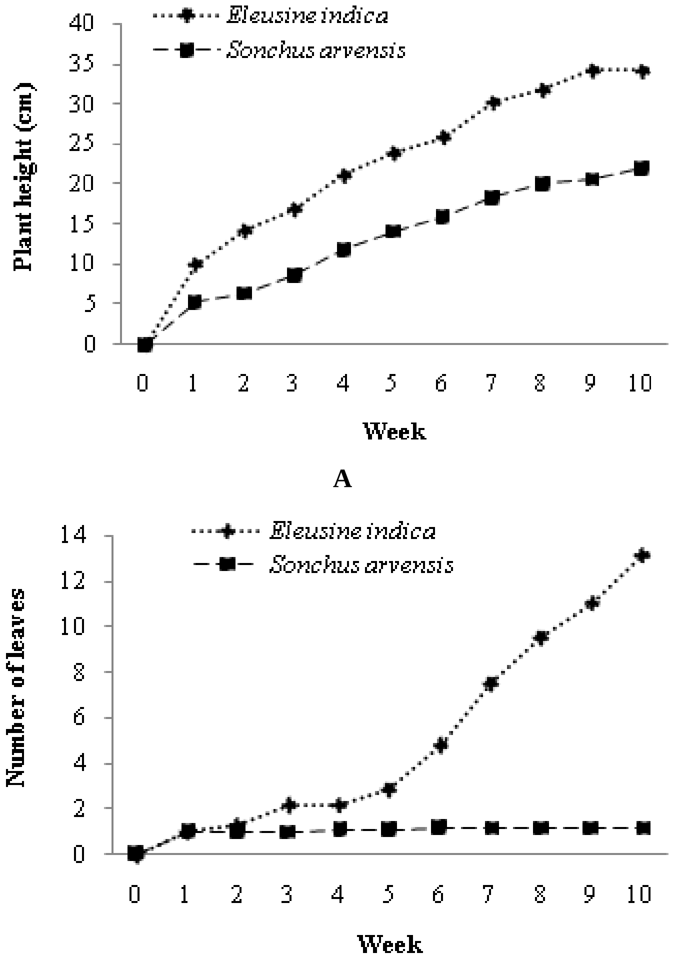
The worldwide average content of Cd in surface soil is less than 1 mg/kg, while in plants levels between 5-30 mg/kg dry weight are toxic (Mulligan et al., 2001). Higher values reflect anthropogenic impact such as long-term use of phosphate fertilizers, sewage sludge application and smelter dust spreading (Salt et al., 1998). Cadmium, lead and mercury are joined together as the big three heavy metals that have a high level of human health hazards (Widaningrum et al., 2007). According to Waseem et al. (2014), Cd is one type of heavy metals that is considered as xenobiotik because it has a minimalist role nearly so useful in the body, even very dangerous because it involves toxic metals and harmful to animals including humans and plants. This study site was horticulture centre, mainly vegetable, with a high intensity of fertilizer uses. The high use of fertilizers, primarily phosphate contributes greatly to the Cd pollution. Tresnawati et al. (2014) reported that plants fertilized with phosphate (SP-36 and NPK) in a prolonged period of time can trigger accumulation of Cd in the soil. The result of measurement of the Cd content has reached 4.22 mg/kg, indicating that it has exceeded the allowable threshold. The threshold value of cadmium in soils is below 2 mg/kg (Abdurachman, 2003), while according to Alloway (1995), the critical limits of Cd in soils is 3 mg/kg. The normal amount of cadmium in the soil should be below 1 mg/kg (Nopriani, 2011). In addition to phosphate pesticide use, especially fungicides also can raise the cadmium content in the soil. Lahuddin (2007) reported that pesticides also contain 0,018 ppm of cadmium, rock phosphate may contains 0-500 mg/kg cadmium.

Growth performance of remediator plants

The research results showed that the two plant species grown in Cd contaminated soil were able to grow well. It means that *E indica* and *S. arvensis* have potency as phytoremediation agents. *E indica* can absorb and accumulate heavy metals (Cd) in their roots (Garba et al., 2011), and serve as Cd stabilizer in the soil; and *S. arvensis* was a good candidate for Pb phytoremediation (Surat et al., 2008). Because of their capability to tolerate or thrive on the toxic metalliferous substrate, they can growth well on heavy metal contaminated soil, especially Cd.

The existence of Cd in soil will affect the plant growth and plant characteristics. Cd provokes changes in both the physiological and morphological characteristics of plants, reducing growth and affecting their assimilatory capacity, water balance, and provoking structural changes (Lux et al., 2004) as well as disturbances of nutrient uptake and distribution. The observations of plant height and number of leaves of the two plant species are presented in Figure 1.

Measurement of plant growth was done in the time of planting period. The measured parameters were plant height and number of tillers (Figure 1). The results showed that *E. indica*, and *S. arvensis* were able to grow well in Cd contaminated soil, which is shown by the plant height and leaves number, even though *E indica* has more tillers number than *S. arvensis*. These differences are associated with plant character. *E indica* is a plant species having high adaptability. *E indica* had a height between 12 - 85 cm. This morphological feature was quite different from the *E indica* which grew Ibadan, Nigeria. In this place, the height of *E indica* acquired a height range of 30 to 62 cm (Sharma, 1984). The same with the *E indica* grew at Ibadan, at study site, *E indica* also has massif rooting that can strengthen the growth, thus *E indica* grew well and have significant number of tillers. The growth performance of plant showed that the two plant species (*E indica* and *S. arvensis*) exhibit a high tolerance to Cd contaminated soil. This was indicated by no inhibition of plant growth as well as physical damage that showed symptoms of toxicity.



A

B

Figure 1. Plant height (A) and number of leaves (B)

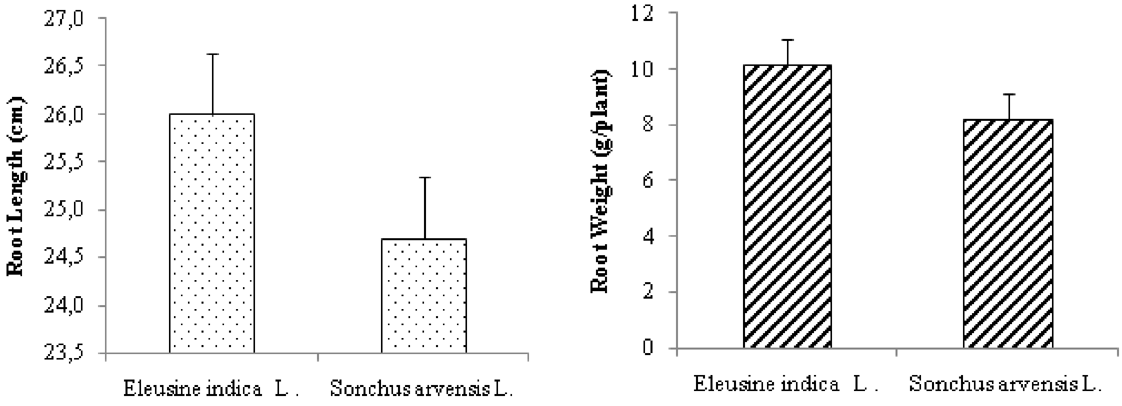
Plant growth is usually reflected by the increase of biomass including dry weight of roots and canopy. The existence of Cd in the soil at high concentration that exceeds a threshold generally can inhibit plant growth. Cadmium will restrict the plant growth and change the plant structure. Plants genetically have the ability to tolerate and adapt to high content of metal elements in soil to survive. Certain plants that are included in metal accumulator plants develop some effective mechanisms to tolerant of high levels of metals in the soil. Plants possess highly specialized mechanisms to stimulate metal bioavailability in the rhizosphere, and to enhance uptake into their roots. Root exudates have an important role in the acquisition of several essential metals.

The results of the observation in the growth of the root length and root weight on plant species presented in Figure 2 show that the highest growth of the root length and root weight were produced by *E.indica*. Both tested plants (*E indica* and *S. arvensis*) were seen almost no difference in root length parameter which was about 24-60 cm. Similarly, the root weight of *E indica* was 10.11 g/plant, while that of *S. arvensis* was 8.20 g/plant. This shows morphological differences of both plants. *E indica* is a grass plant that is able to produce more roots than *S. arvensis* which is a broadleaf plant. Some grass species have been documented to exude from their roots a class of organic acids called siderophores (mugeneic and avenic acids), which were found to significantly enhance the bioavailability of soil-bound iron and possibly zinc (Cakmak et al., 1996).

It has been reported that most grass specie are known to concentrate heavy metals in the roots, with only very low translocation to the shoot (Speir et al., 2003). Morphological differences of each plant will have an effect on the ability of tolerance in the absorption of elements.

According to Marchial et al. (1996), the differences in the behaviours of species maybe due to genetic or physiological features such as the existence of blockers in roots promoting Cd allocation to the cell walls and apoplastic structures. Plants that are able to adapt to the heavy metal contaminated environment, generally have the ability to accumulate heavy metals. Heavy metals are absorbed in the high amount subsequently translocated to the roots, stems and leaves. Plants possess highly specialized mechanisms to stimulate metal bioavailability in the rhizosphere, and to enhance uptake into their roots.

Root exudates have an important role in the acquisition of several essential metals. Alberto and Sigua (2013) pointed out that in general, the success of phytoremediation technology depends on several factors. First, the plant should produce enough biomass as well as absorb high heavy metals. Second, plants growth should be responsive towards heavy metal accumulation in the plant tissue.



A **B**

Figure 2. Root length (A) and root weight (B) of remediator plants

According to Gomes et al. (2012), dry weight (DW) production was negatively affected by Cd concentrations. Cd had inhibiting effects on growth up to concentrations of 45 µmol Cd; at higher concentrations, dry biomass production of both shoots and roots showed only slight changes. In some cases, the increase of biomass will decrease the total concentration of metals in plant tissues, but this allows for a larger amount of metal to be accumulated as a whole. Plants with specific genetic characteristics are able to grow and accumulate heavy metals. Certain types of plants will be able to maintain the ability of heavy metal accumulation through absorption and precipitation (Alberto and Sigua, 2013). This experiment showed that both plants are having relatively similar characteristics, even though *E indica* is taller than *S. arvensis. E indica* belongs to grass plant, so they have more capability to grow compared with *S. arvensis*. The results also showed that both of plant species were very tolerant plants in Cd contaminated soil. The capability of plants to adapt in contaminated area will affect their capability in absorbing heavy metals.

Accumulation and reduction of Cd

Plants that are able to accumulate heavy metals are indicated to have the ability as an agent of phytoremediation. The capability of *E. indica*, and *S. arvensis* in absorbing cadmium after 3 months planted were presented in Figure 3. Generally, root parts will absorb greater Cd than the shoot part. Cd phytotoxicity was noticeable in the roots, which showed greater decreases in biomass production than the shoots. In addition to coming directly in contact with substrate Cd, roots showed higher Cd contents than shoots (Gomez et al., 2012).

Sorption of Cd on plant ranged from 0.6 to 1.1 mg/kg. This heavy metal was accumulated in the root and shoot, i.e. 1.08 and 0.73 for *E. indica* and 0.99 and 0.61 mg/kg for *S. arvensis*, respectively. On average, sorption of Cd in the shoot and the roots parts of *E indica* was higher than Cd sorption on *S. arvensis* and root parts, was also higher in accumulating Cd than the shoot parts. However, the differences between two plant; *E indica* and *S. arvensis* in absorbing Cd in their body is not significant. This is shows that .*E indica* has the ability of living high tolerance so that it is able to accumulate higher Cd. The high level and poor or low translocation of the elements to the shoots could be due to sequestration of the elements in the vacuoles of the root cells to render them non-toxic that may be a natural toxicity response of the grass plant.

It has been reported that one of the mechanisms by which uptake of metal occurs in the roots may include binding of the positively charged toxic metal ions to negative charges in the cell wall (Gothberg et al., 2004). The big difference between root and shoot concentrations indicates an important restriction of the internal transport of Cu, Cd, Ni and Zn from roots to shoot, resulting in higher root concentrations rather than translocation to shoots. Low transport of these metals to shoot may therefore be due to saturation of root metal uptake, when the internal metal concentrations are high.

The accumulation and level of the elements in either the roots or shoots of the grass plant does not actually read the hyperaccumulating potential. It is the metal transfer coefficients in term of enrichment coefficient or translocation Factor (TF) that determines the hyperaccumulating potentials of plant species under experiment. Translocation factor is a measure of the ability of plants to transfer accumulated metals from the roots to the shoots.

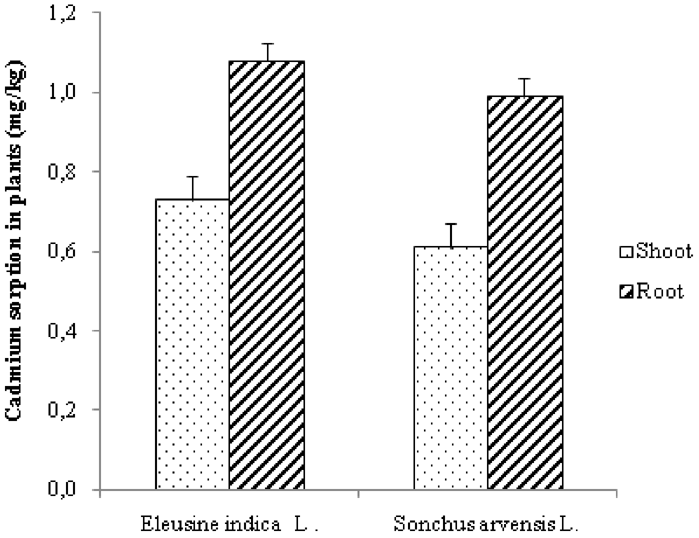


Figure 3. Cd absorption on shoot and root part of remediator plants

The enrichment coefficient (EC) was used to evaluate the ability of plant to accumulate heavy metals in the root. The plants will be categorized as hyperccumulator plants if the translocation factor from roots to shoot was greater than one (Gabbrielli et al., 1991). However, in this experiment the TF ratios both of plants were smaller than one (0.7 and 0.6 for *E. indica* and *S. arvensis*, respectively), and the enrichment factor was 0.3 for both of plant types. This indicates that both plants are not hyperaccumulator plants but they can be categorized as remediator plants. Differences in the ratio of heavy metals in the canopy and roots in both species showed differences in the mechanisms of each species in the process of translocation of heavy metals from the roots to shoot. Plants develop some effective mechanisms to tolerate high levels of metals in the soil. Accumulator plant does not prevent metals to get into roots but develop specific mechanisms to detoxify heavy metals and accumulating them in the cell. This mechanism allows the bioaccumulation of metals in high concentrations. High accumulation in plants reflects the high concentration of metals in rhizosphere. Patra and Sarma (2000) indicated that there is a link between the levels of heavy metal contamination in the soil with that absorbed by plants. Elevated levels of cadmium in the soil will give an impact on increasing the uptake of cadmium by plants that are accumulated in the roots or shoots. Accumulation occurs because there is a tendency of heavy metals to form complexes with inorganic substances found in the body of the organism. This research showed that the two plant species accumulated more Cd in the roots than in the shoot. *E. indica* accumulated Cd higher than *S. arvensis*. The difference is due to the difference of rooting system (Figure 4). *E indica* has a massif rooting system that makes the plant enables to do phytoextaction of heavy metals.


Eleusine indica *Sonchus arvensis*

Figure 4. *E. indica* and *S. arvensis* rooting systems

In the process of phytoextaction, heavy metals are absorbed by plant roots and translocated into the shoot to be processed or disposed when the plants are harvested. In phytoremediation technique, the root zone is of special interest. The contaminants can be absorbed by the root to be subsequently stored or metabolized by the plant. Degradation of contaminants in the soil by plant enzymes exuded from the roots is another phytoremediation mechanism. Generally, hyperaccumulator plants adapt to heavy metal contaminated soil through the accumulation of heavy metals in the root system, the translocation of heavy metals from the root to the shoot, as well as through sequestration and detoxification of heavy metals in the leaves.

Physiological and molecular characteristics determine the level of accumulation of heavy metals in plants (Tian et al., 2009; Oomen et al., 2009). Cd is a heavy metal that is most difficult to be absorbed. As a result, Cd is more readily available to plants compared to other heavy metals, such as Cu, Pb and Cr (Gomes et al., 2001). This means that the plant is easier to absorb Cd than other metals such as Cu Pb, and Cr because Cd is weakly bound in soil. Furthermore, Cd that is absorbed by the roots is generally accumulated in the root. The heavy metals taken up by the grass plant *E indica* can be arranged in the following order: Zn> Ni> Cu>Se>Pb>Cd in the root while in the shoot they can be arranged as Zn>Cu>Pb>Ni>Se>Cd (Garba et al., 2011). However, on the vegetables crops such as lettuce (*Lactuca sativa* L.) Cd is more accumulated in the leaves than in the roots. The overall results showed that *E.indica* and *S. arvensis* were able to reduce Cd concentration in the soil by 57.11% and 35.84%, respectively (Figure 6).

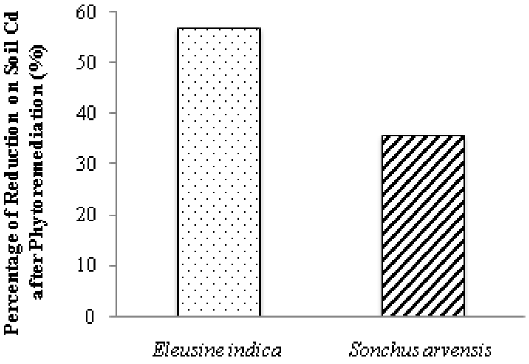


Figure 6. Percentage of Cd reduction in each plant

Based on the high reduction of Cd concentration in the soil indicates that the two plant species can be used as agents for phytoremediation of Cd contaminated soils. The high ability of plants to absorb heavy metals is associated with the massive rooting system. The plants that have massive rooting systems are capable of reducing heavy metal concentration in soils. According to Alberto and Sigua (2013), plants that are able to decontaminate contaminated soil have some characters, i.e. 1) the occurrence of plant uptake from soil or fluid that contaminated to the root, 2) bind the contaminants into the tissues of plants, and 3) carries contaminants from the root to the top of the plant as well as prevent or inhibit the contaminants from the soil.

Conclusion

Two wild vegetation species of *E indica* and *S. arvensis* were able to grow well in Cd contaminated soil in the horticulture centre of Pujon sub district. Cd accumulation in plant roots was higher than in plant shoot. *E indica* accumulated Cd higher than *S. arvensis*, as well as being able to reduce Cd content by 57.11% and 35.84%, respectively.

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