LEAD AND MERCURY UPTAKE IN DOMINANT MACROPHYTES OF BALILI RIVER, BENGUET, PHILIPPINES

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Abstract: Lead and mercury contamination have been reported in several sites in the Philippines including Balili River, the drainage of Baguio City and La Trinidad, Benguet. Amidst this, the river is still vital to the community as irrigation source, thus the immediate need for its rehabilitation. The study has assessed the potential of common macrophytes growing along the riparian such as *Alternanthera sessilis*, *Cynodon dactylon*, *Solanum americanum* and *Tithonia diversifolia* for lead and mercury uptake. *Cynodon dactylon* and *Solanum americanum* registered the highest Pb concentrations in all plant organs but they differ in their translocation (TF) capabilities. *Cynodon dactylon* has higher Pb in its stem qualifying it as phytoextractor while *Solanum americanum* has low translocation in its shoots qualifying it only as a phytostabilizer. *Alternanthera sessilis* and *Tithonia diversifolia* have much lower Pb uptake showing the metal excluding capacity of these plants for lead. In terms of Hg uptake, *Alternanthera sessilis* and *Solanum americanum* have higher absorption in their roots while *T. diversifolia* and *Cynodon dactylon* have higher values in their shoot organs, particularly in leaf. Consequently, *Tithonia diversifolia* and *C. dactylon* qualify as phytoextractor for Hg having a bioaccumulation factor (BCF) and TF values > 1. On the hand, *Alternanthera sessilis* and *Solanum americanum* at BCF > 1 and TF < 1 qualify as phytostabilizers for Hg. These results show that macrophytes in Balili River, even if they differ in their heavy metal uptake, could be tapped for the phytoremediation of its heavy metal contamination.

Keywords: *Alternanthera sessilis*, Balili River, *Cynodon dactylon*, lead, mercury, *Solanum americanum*, *Tithonia diversifolia*

Introduction:

Heavy metal pollution has become a serious health concern in recent years, because of industrial and agricultural development. The health effects of heavy metals have been known since ancient history (Nriagu 1996) and their toxicity and / or carcinogenicity have been reported in numerous studies (Tong et al. 2000; Neff 1997). In brief, they may cause kidney, lung, nervous system, and skeletal damages, such as itai-itai disease (Nishijo et al. 1995; Staessen et al. 1999). Diminished intellectual capacity, gastrointestinal symptoms, coronary heart disease, a variety of cancers such as renal,

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skin and bladder cancers, and even death have been also associated with chronic and/or acute exposures to heavy metals (Järup 2003; Kolonel 1976; Lanphear et al. 2005).

Heavy metal toxicity is potentially dangerous because of bio-accumulation through the food chain (Aycicek et al. 2008). Unlike plant nutrients, heavy metals break down very slowly; without remediation they can exist in the environment for a very long time. Among the heavy metals, lead (Pb), cadmium (Cd) and mercury (Hg) are the most likely to pose the greatest health risk to humans. Lead poses the greatest concern among heavy metals because it is the most common contaminant and is most likely to exceed health based guidance values (McBride et al. 2014). Lead does not degrade and can remain in the soil for thousands of years. Lead accumulates on the top 1-2 inches of the soil as it binds tightly to soil particles and organic matter (Shacklette and Boerngen 1984). On the other hand, mercury contamination of land, water, and air has been a global issue for many years (Fitzgerald and Clarkson 1991). Mercury has no biological function in humans and exposure to high Hg concentrations is a potential hazard (USEPA 1997). All forms of Hg are toxic, but organic Hg compounds such as methyl-Hg (CH_3Hg) are the most toxic (Ullrich et al. 2001).

Lead and mercury contamination have been reported by DENR-CAR in Balili River, La Trinidad, Benguet, Philippines. This river is an important irrigation source in La Trinidad Valley, dubbed as the ‘Salad Bowl of the Philippines’. Several possible sources of lead exist in Balili River such as paints (from old buildings or discarded residues), furniture and car shops, vehicular emissions that settles on the water or have been carried by run-off, and many others. On the other hand, the recorded Hg in the river most likely comes from inorganic fertilizers and occasional mine tailings clean-up and Hg-based gold recovery activities (Napaldet and Buot 2019).

Situated in a developing country, conventional physico-chemical clean-up is not an option for the heavy metals of Balili River thus, alternative options like phytoremediation are being sought. Because it is a natural process, phytoremediation is now an emerging potential effective technology in reclaiming contaminated areas because of its cost effectiveness, aesthetic advantage, “green clean” approach and long-term applicability to a wide range of contaminants (Tulod et al. 2012). The aquatic macrophytes growing in the river could offer the most viable solution to its Pb and Hg contamination. However, there is a need to first characterize the heavy metal uptake in aquatic macrophytes of the river.

Baker and Walker (1990) categorized plants into three groups based on their strategies for growing on metal-contaminated soils, namely, metal excluders, indicators and accumulators or hyperaccumulators. Plants that effectively limit the levels of heavy metal translocation within them and maintain relatively low levels in their shoot over a wide range of soil levels are considered metal excluders; however, they can still contain large amounts of metals in their roots. On the other hand, metal indicator plants accumulate metals in their above-ground tissues and the metal levels in the tissues of these plants generally reflect metal levels in the soil, however, continued uptake of heavy metals cause these plants to die-off. Lastly, plant species that concentrate metals in their above-ground tissues to levels far exceeding those present in the soil or in the non-accumulating species growing nearby are considered metal accumulators (hyperaccumulators). These plants could be ideal for phytoremediation since they are capable of extracting heavy metals from soils and concentrate them in their shoots.

Materials and methods:

Study area

Balili River is an important water resource of Baguio City and La Trinidad, Benguet. However, the river suffers from excessive pollution which is usually blamed on the densely populated city of Baguio, its headwaters. It was included in the DENR's 2003 Pollution Report as one of the 15 "biologically dead" rivers among the 94 principal river basins in the country. The river has a total length of 23.81 kilometers traversing the city of Baguio, the municipalities of La Trinidad and Sablan before entering the province of La Union (Aro 2011; Palangchao 2011).

Analysis of Mercury Lead (Pb) and (Hg)

Common macrophytes growing along Balili River were identified. These include *Alternanthera sessilis*, *Cynodon dactylon*, *Solanum americanum*, and *Tithonia diversifolia*. Plant samples of these species were harvested along the littoral zone of the main stream from km 5 to km 6 stretch of the river, selecting samples nearest to the water channel. These samples were derived from several randomly selected individuals then segregated into major plant organs. A composite sample of at least 100 g was derived per plant organ. The samples were chopped into the required small sizes then submitted to DOST-CAR laboratory, an ISO-certified laboratory, for Pb and Hg determination. Lead was analyzed by atomic absorption spectrophotometry after microwave digestion while mercury by thermal decomposition amalgamation and atomic absorption spectrophotometry.

Additionally, the soil on the river's riparian was also analyzed for Pb and Hg. Soil samples were gathered from the same area where the plant samples were collected. Following the recommended method by Baker et al. (1994) and Gonzalez and Gonzalez-Chavez (2006), composite soil samples were randomly derived along the littoral zone at the 0-25 cm depth that was considered as the plant root zone. These samples were air-dried then submitted to same DOST-CAR laboratory for Pb and Hg determination.

Bioconcentration Factor (BCF) and Translocation or Transfer Factor (TF) Determination

To evaluate the metal accumulation efficiency of the selected macrophytes, bioaccumulation coefficient factor (BCF) and translocation factor (TF) were calculated. Bioconcentration factor is the ratio of the roots' total metal concentration to that in the soil (Elkhatib et al. 2001; Gonzalez and Gonzalez-Chavez 2006; Yoon et al. 2006). It was computed as follows:

$$BCF = [M]roots / [M]soil$$

where
[M]roots is the total metal concentration in the roots, and
[M]soil is the total metal concentration in the soil, and wherein for this particular study the metal refers to Pb and Hg.

On the other hand, the translocation or transfer factor is the ratio of the shoots' total metal concentration to that in the roots (Mocko and Waclawek 2004; Yoon et al. 2006; Sanghamitra et al. 2012). It was computed as follows:

$$TF = [M]leaves / [M]roots$$

where
[M]leaves is the total Pb or Hg concentration in the leaves, and
[M]roots is the total Pb or Hg concentration in the roots.

BCF and TF are used to estimate a plant's potential for phytoremediation (Yoon et al. 2006). BCF is used to estimate a plant's ability to accumulate the metal in the roots while TF is used to estimate a plant's ability to translocate metals from the roots to above-ground parts or the internal metal transportation (Nouri et al. 2009). According to Yoon et al. (2006), plants exhibiting BCF and TF values of less than one are not suitable for phytoextraction. On the other hand, plants with both BCF and TF greater than one (BCF

> 1, TF > 1) have the potential to be used in phytoextraction while plants with bioaccumulation factor greater than one and translocation factor less than one (BCF > 1 and TF < 1) have the potential for phytostabilization. Kabata-Pendias (2011) added that a hyperaccumulator plant should have BCF > 1 or TF > 1, as well as total accumulation > 1000 mg kg^{-1} of Cu, Co, Cr or Pb, or > 10000 mg kg^{-1} of Fe, Mn or Zn.

Results and discussion:

Lead Uptake in Dominant Macrophytes of Balili River

Dominant macrophytes of Balili River differ in their lead content, which indicated that they differed in their capacities for Pb uptake (Fig. 1). *Cynodon dactylon* and *Solanum americanum* register the highest Pb concentrations in all plant organs that is much higher than soil substrate. Consequently, *C. dactylon* and *S. americanum* have the highest BCF values (Tab. 1) and are higher than 1. However, *C. dactylon* and *S. americanum* differ in their TF capabilities for the absorbed lead that can be readily gleaned from Figure 1. *Cynodon dactylon* has higher Pb in its stem, netting a TF value of 1.14. High TF denotes translocation of the metal from the roots to the shoots. This means that *C. dactylon* has BCF > 1 and TF > 1, qualifying it as a phytoextractor. This result is significant since most plants accumulated lead (approximately 95% or more) in their roots, and only a small fraction is translocated to aerial plant parts, as reported in *Vicia faba*, *Pisum sativum*, *Phaseolus vulgaris*, *V. unguiculata*, *Nicotiana tabacum*, *Lathyrus sativus*, *Zea mays* and *Avicennia marina* (Pourrut et al. 2011). Moreover, our result validates the findings of Río-Celestino et al. (2006) that found *C. dactylon* to be efficient phytoextractor of Pb and Zn under experimental condition. However, the concentration of Pb in *C. dactylon* in this study is much higher than those reported by Soleimani et al. (2009) at 10-15 mg/L in root and 8-12 mg/L in shoot.

Figure no. 1 Lead concentration in major plant organs of the dominant macrophytes of Balili River

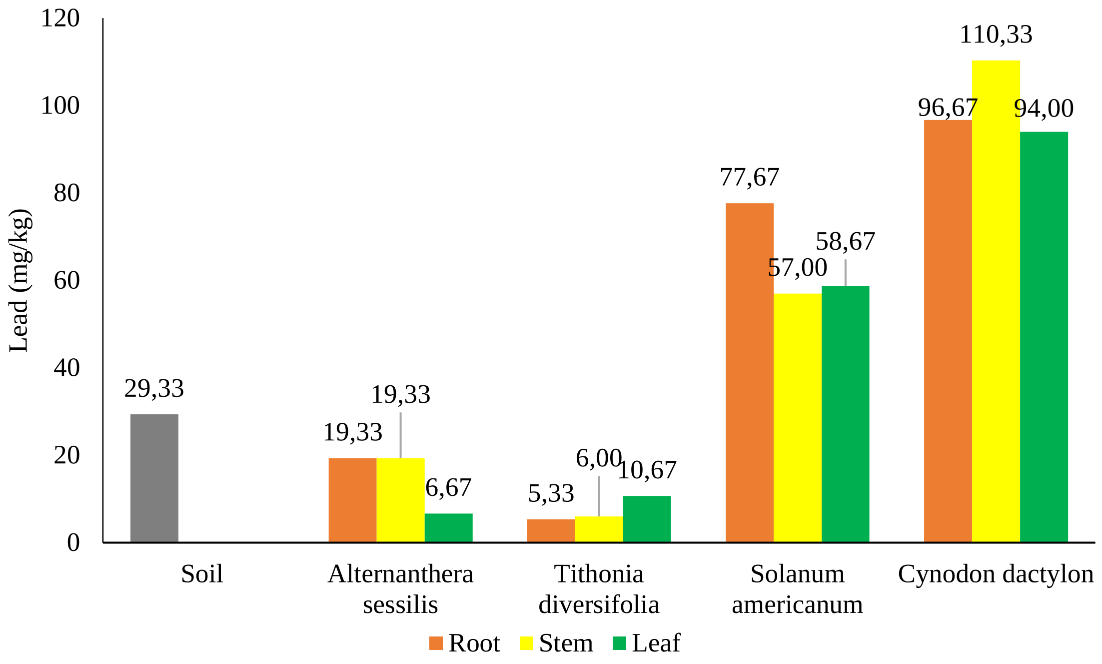


Table no. 1 Bioaccumulation coefficient factor (BCF) and translocation factor (TF) of dominant macrophytes of Balili River for selected heavy metals

Plant Species	Lead (Pb)			Mercury (Hg)		
	BCF	TF_{stem}	TF_{leaf}	BCF	TF_{stem}	TF_{leaf}
Alternanthera sessilis	0.66^{a}	1.00^{a}	0.35^{a}	2.21^{c}	0.59^{b}	0.34^{a}
Tithonia diversifolia	0.18^{a}	1.20^{a}	2.09^{b}	1.76^{b}	1.18^{c}	1.75^{c}
Solanum americanum	2.69^{b}	0.74^{a}	0.76^{a}	2.46^{c}	0.30^{a}	0.56^{a}
Cynodon dactylon	3.31^{b}	1.14^{a}	0.97^{a}	1.27^{a}	1.10^{c}	1.40^{b}

Note: Means with the same letter in a column are not significantly different at 0.05 Tukey's HSD

There is higher translocation of lead in the stem of *C. dactylon* than on its leaf. Also, the stem had significantly higher lead concentration (p = 0.00) indicating that its heavy metal uptakes are primarily stored in the stem. This is readily conceivable because of the wide span of parenchymatous ground tissues in the stems, which primarily function for storage of substances including heavy metals. These findings on the high Pb concentration and high TF values in *C. dactylon*'s shoot are disturbing since this grass is regularly being harvested as fodder since it is higher than maximum acceptable Pb level for forage set in EU Regulation No. 744/2012 (Adamse et al. 2017) at 30 mg/kg. There is a need to immediately alert the local authorities on these results.

Solanum americanum has low translocation of lead in its shoots. Nonetheless, its high BCF would qualifying it as a phytostabilizer. This trend is consistent with findings of Varun et al. (2015) which found higher Pb accumulation in the plant's root that in its shoots. However, much higher Pb concentrations are recorded for *S. americanum* in this study. The maximum lead in Varun et al.'s study is at 32.6 mg/L in roots and 6.1 mg/L in shoots at 250 mg/L experimental soil concentration, which is even much higher than the soil Pb in our study. This difference in performance of same plant species at different environmental conditions would suggest that there are several factors affecting the plant's ability for heavy metal uptake. In this study, the higher Pb accumulation in *S. americanum* and *C. dactylon* could be attributed to the high organic matter and high water content of the riparian soil. These two factors have been identified to increase lead absorption in plants (Traunfeld and Clement 2001).

On the other hand, *Alternanthera sessilis* and *Tithonia diversifolia* have much lower Pb in their major organs. It is also lower that the soil media. Consequently, they have low BCF values. Interestingly, *Tithonia diversifolia* has the significantly highest TF value in leaf but its implication is negated by its low uptake. According to Olivares (2003), Pb rout of entry in *T. diversifolia* is more of foliar absorption than root uptake. This could explain the higher Pb concentration in the plant's leaf than its roots.

The low uptake in *A. sessilis* and *T. diversifolia* show that these plants are metal excluder for lead and are not recommended in phytoremediation. This result contradicts the findings of Mazumdar and Das (2015) and Li et al. (2008) that found *A. sessilis* to be a good phytoremediator of heavy metals such as Pb, Zn, Fe and Mg. Moreover, it contradicts the findings of Olivares (2003) which found *T. diversifolia* to absorb high amounts of Pb in its roots at 34.94 ppm and in its leaf at 120.79 ppm. Our result is more comparable with Ayesa et al.'s (2018) finding which determine *T. diversifolia*'s Pb uptake at 2.37 ppm.

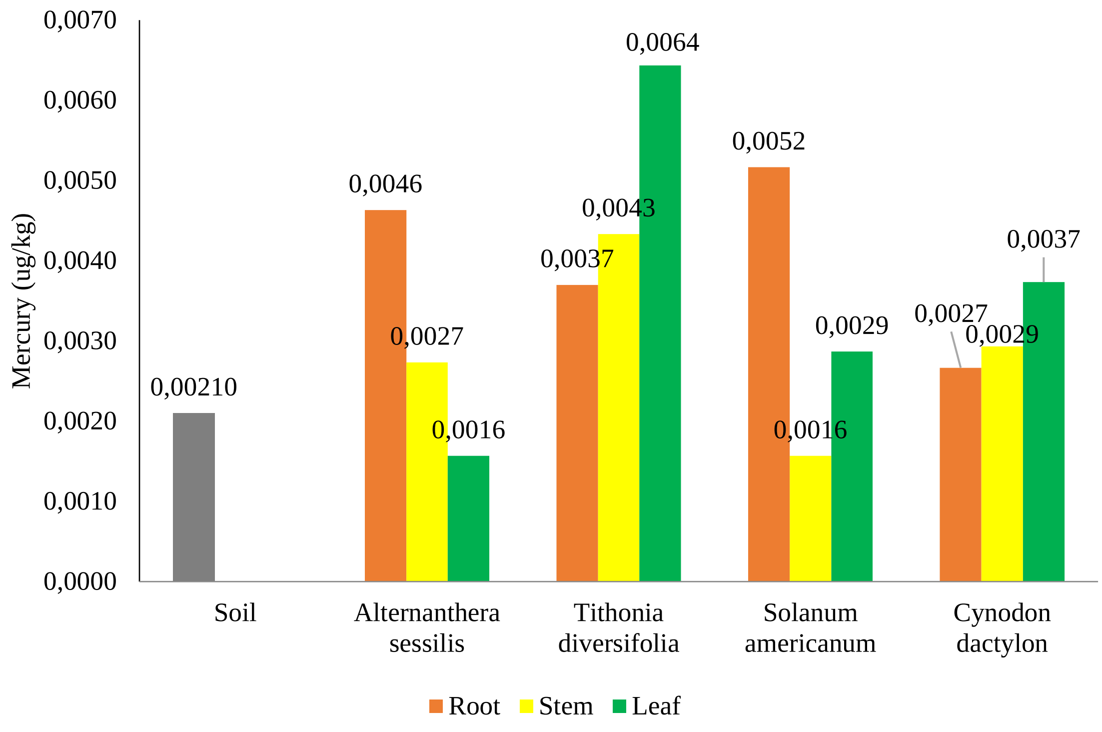
An earlier assessment of Napaldet and Buot (2020) on lead and mercury in *Amaranthus spinosus*, *Eleusine indica* and *Pennisetum purpureum* at same Balili River showed much lower lead concentrations in soil and in plant organs. Much higher Pb is recorded in riparian soil in this study at 29.33 mg/L compared to the earlier assessment at

2.4 mg/L only. The difference could be attributed to the time of sampling wherein the earlier assessment was conducted from March to April while this study was conducted on June, the onset of rainy season in the locality. At this time, run-off coming from the nearby urban areas of Baguio and La Trinidad are loaded with several pollutants, including lead.

Mercury Uptake in Dominant Macrophytes of Balili River

Figure 2 presents the Hg concentrations in major plants organs of the sampled macrophytes. The figure readily shows that Hg concentrations in the plant roots were significantly ($p = 0.00$) higher than in the soil. *Alternanthera sessilis* and *S. americanum* have higher Hg absorption in their roots while *T. diversifolia* and *C. dactylon* have higher values in their shoot organs, particularly in their leaf. Consequently, *A. sessilis* and *S. americanum* have high BCF values but low TF values (see Tab. 1) while *T. diversifolia* and *C. dactylon* have lower BCF values but higher TF values. Following the criteria set in Kabata-Pendias (2011), *T. diversifolia* and *C. dactylon* qualify as phytoextractors for Hg having BCF > 1 and TF > 1. *Alternanthera sessilis* and *S. americanum*, on the hand, at BCF > 1 and TF < 1 are phytostabilizers for Hg. The Hg uptake of *C. dactylon* in this study contrast with Weaver et al.'s (1984) study which recorded much higher uptake in roots than on its leaf.

Figure no. 2 Mercury concentration in major plant organs of the dominant macrophytes of Balili River



The high translocation of Hg in shoots of *T. diversifolia* and *C. dactylon* is quite interesting because it differs from other studies that have generally found low translocation of Hg. However, the difference could be attributed to the low Hg concentration in the soil media of our study compared to other studies and not really due to the translocation prowess of the plants investigated in the study. Sas-Nowosielska et al. (2008) recorded minimal Hg translocation from roots to shoots in *Festuca rubra*, *Poa pratensis*, *Armoracia lapathifolia*, *Helianthus tuberosus* and *Salix viminalis*. Additionally, Hussain et al. (2010) recorded low Hg translocation in *Vigna mungo* and by Moreno et al. (2008) in *Brassica juncea*. Pourrut et al. (2011) explained that the minimal translocation of Hg could be due to immobilization by negatively charged pectins with in the cell wall, precipitation of insoluble lead salts in intercellular spaces, accumulation in plasma membranes, sequestration in the vacuoles of rhizodermal and cortical cells and physical barrier provided by the root endodermis, though the mechanism is not yet fully understood.

On the other hand, our result is more consistent with the study of Rodriguez et al. (2005) which recorded significant translocation (TF > 1) of Hg in *Triticum aestivum*, *Hordeum vulgare* and *Lupinus luteus*. Cavallini et al. (1999) and Skinner et al. (2007) also recorded significant translocation of Hg in *Pistia stratiotes* and in *Triticum durum*. In *Triticum durum*, Hg was found incorporated in leaf epidermal and stomatal cell walls and on parenchyma cell nuclei. This could most likely be the same in the case of *T. diversifolia* and *C. dactylon*.

Comparing the results in this study with the earlier assessment of Napaldet and Buot (2020) in the same Balili River, the macrophytes in this study have higher Hg uptake than that of *Amaranthus spinosus* and *Eleusine indica* but much lower than *Pennisetum purpureum*. Factoring in biomass, it would appear that *P. purpureum* would still be the best candidate for phytoremediation of Hg in the river.

These results shows that different macrophytes in Balili River differ in their heavy metal uptake. This is consistent with the findings of several studies that heavy metal uptake could be species specific (Freitas et al. 2004; Nouri et al. 2009; Nazareno and Buot 2015). Thus, assessment of heavy metal uptake of few plants is not enough but should be as many plants as possible, though subject to availability of funds.

Conclusions:

The aquatic macrophytes used in the study differed in their capacities for Hg and Pb uptake. *Cynodon dactylon* and *S. americanum* registered the highest Pb concentrations in all plant organs. Consequently, *C. dactylon* and *S. americanum* have the highest BCF values that are higher than 1 but they differ in their translocation (TF) capabilities. *Cynodon dactylon* has higher Pb in its stem qualifying it as phytoextractor while *S. americanum* has low translocation in its shoots qualifying it only as a phytostabilizer. *Alternanthera sessilis* and *T. diversifolia* have much lower Pb uptake showing that these plants are metal excluders for lead. Interestingly, *T. diversifolia* has the significantly highest TF value in leaf but its implication is negated by its low uptake. This contradicts findings of other studies that find *A. sessilis* and *T. diversifolia* to be good phytoaccumulators of Pb.

In terms of Hg uptake, *A. sessilis* and *S. americanum* have higher absorption in their roots while *T. diversifolia* and *C. dactylon* have higher values in their shoot organs, particularly in leaf. Consequently, *T. diversifolia* and *C. dactylon* qualify are phytoextractors for Hg having a bioaccumulation factor (BCF) and TF values >1. On the hand, *A. sessilis* and *S. americanum* at BCF > 1 and TF < 1 qualify as phytostabilizers for Hg. These results shows that different macrophytes in Balili River differ in their heavy metal uptake which is consistent with the findings of several studies asserting that heavy metal uptake could be species specific. Nonetheless, these macrophytes could be tapped for the phytoremediation of the river’s heavy metal contamination.

Rezumat:

ABSORBȚIA PLUMBULUI ȘI MERCURULUI ÎN PLANTELE DOMINANTE DIN RÂUL BALILI, BENGUET, FILIPINE

Contaminarea cu plumb și mercur a fost raportată în mai multe locuri din Filipine, inclusiv râul Balili, canalizarea orașului Baguio și La Trinidad, Benguet. Pe fondul acestui fapt, râul fiind încă vital pentru comunitate ca sursă de irigații, se impune deci nevoia imediată de reabilitare a acestuia. Studiul a evaluat potențialul de creștere a macrofitelor comune de-a lungul malurilor râului, cum ar fi *Alternanthera sessilis*, *Cynodon dactylon*, *Solanum americanum* și *Tithonia diversifolia*, în condițiile absorbției plumbului și mercurului. *Cynodon dactylon* și *Solanum americanum* au înregistrat cele mai mari concentrații de Pb în toate organele plantelor, dar diferă prin capacitățile lor de translocație (TF). *Cynodon dactylon* are Pb mai mult în tulpină, calificându-l drept fitoextractor, în timp ce *Solanum americanum* are translocație scăzută în lăstari, calificându-l doar ca fitostabilizator. *Alternanthera sessilis* și *Tithonia diversifolia* au o absorbție de Pb mult mai mică, indicând capacitatea de excludere a plumbului de către aceste plante. În ceea ce privește absorbția Hg, *Alternanthera sessilis* și *Solanum americanum* au o absorbție mai mare în rădăcini, în timp ce *Tithonia diversifolia* și *Cynodon dactylon* au valori mai mari în organele vegetative, în special în frunze. În consecință, *Tithonia diversifolia* și *Cynodon dactylon* se califică ca fitoextractori pentru Hg având un factor de bioacumulare (BCF) și valori TF > 1. Pe de altă parte, *Alternanthera sessilis* și *Solanum americanum* la un BCF > 1 și TF < 1 se califică ca fitostabilizatori pentru Hg. Aceste rezultate arată că macrofitele din râul Balili, chiar dacă diferă prin absorbția lor de metale grele, ar putea fi exploatate pentru fito-remedierea contaminării cu metale grele.

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