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ARBES RIVER WATER QUALITY BASED ON HEAVY METAL CONTENT AS WELL AS THE POTENTIAL OF *LIMNOCHARIS FLAVA*, *PISTIA STRATIOTES*, AND *HYDRILLA VERTICILLATA* AS PHYTOREMEDIATOR AGENTS

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Abstract: The Arbes River is one of the drinking water sources for the Ambon City community in Indonesia. However, the river is polluted by B3 heavy metals, which pose a significant health risk and may cause death. The presence of these metals in the river is due to the high activity of the community in using it to fulfil their daily needs. This study was preceded by a survey to assess the condition of the Arbes River and the activities of the surrounding community. Water samples were analysed for B3 heavy metal content, specifically lead (Pb), cadmium (Cd), chromium (Cr), and mercury (Hg), and the potential of *P. stratiotes, L. flava* and *H. verticillata* as phytoremediator agents were analysed. The results showed that the water quality of the Arbes River, as measured by B3 heavy metal content in six segments, exceeded classes III and IV quality standards, making it unsuitable for drinking but potentially suitable for irrigation purposes. Aquatic plants can be used to remediate the B3 heavy metal content in the Arbes River, with *H. verticillata* demonstrating better remediation potential for lead, cadmium, chromium, and mercury than *L. flava* and *P. stratiotes*. Consequently, *H. verticillata* shows promise as a potential phytoremediator agent for remediating B3 heavy metals in the water of Arbes River.

Keywords: Laboratory scale, aquatic plants, remediation, phytochelatins, phytokelamines.

Introduction

The Arbes River is a raw source of drinking water for the community of Ambon City, Indonesia. It is also a tourist attraction, as well as a place for washing, bathing, and disposing of domestic waste. The upstream part of the Arbes River is primarily used for tourist activities and as a bathing and washing location. The middle part of the river is mostly used for domestic waste disposal and washing, while the downstream part is predominantly used for waste disposal.

Uncontrolled community activities in the use of the river have led to waste entry and a potential increase in heavy metal content. Heavy metal waste is generated by illegal mining activities, factory waste, as well as household waste from plastic materials, cans, and fuel residue (Ashraf *et al.*, 2019). Waste classified as B3 heavy metals includes lead, cadmium, chromium, and mercury, which have high toxicity and can cause the death of organisms (Hussain *et al.*, 2017; Khalid *et al.,* 2017). For instance, Minamata disease in Minamata Bay, Japan, is caused by mercury waste, while the itai-itai disease among the Japanese community is caused by consumption of fish contaminated with cadmium (Hava & Kagan, 2021)

Agustiningsih (2012) reported those settlement activities were the primary source of waste load input in the Blukar River, Kendal Regency, leading to poor water quality. Biological oxygen demand waste loads from settlements, agriculture, and industry were 641.75 kg/day, 284.32 kg/day, and 8.23 kg/day, respectively. Additionally, community activities, such as bathing, washing, and defecating, in the Blukar River contributed to the organic pollutant load. Agricultural activities, specifically the excessive use of fertilisers and pesticides, and the failure of fish processing industries to treat their wastewater properly also contributed to the pollutant load in the river (Hussain *et al.,* 2017).

Several efforts can be made to restore the quality of polluted water, such as providing public education on the proper disposal of untreated wastes, applying sanctions for violators of water pollution regulations, and using plants to remediate waste. Arwini (2011) suggested that aquatic plants, such as water hyacinths, can be used as bioaccumulators for lead waste. Meanwhile, the usage of sunflowers with floating systems has the potential to accumulate large amounts of lead (Farid *et al.,* 2017). Stowell *et al.* (2000) stated that aquatic plants can neutralise certain components in the waters and are very useful in the wastewater treatment process. Suriawiria (2003) found that the arrangement of aquatic plants in small beds in a treatment pond is effective as a living filter for liquid waste that is passed through the bed. These studies suggest that the ability of aquatic plants to filter materials can be used as part of liquid waste treatment efforts. Reed (2005) explored a filtration and absorption process using the roots and stems of aquatic plants, as well as a process of ion exchange and absorption on plants exposed to liquid waste.

The use of aquatic plants as phytoremediator agents is essential to overcome water pollution due to their easy handling and natural growth. Water lettuce (*Pistia stratiotes*), yellow bur head (*Limnocharis flava*), and hydrilla (*Hydrilla verticillata*) are plants that commonly inhabit freshwaters with very fast propagation and dispersal rates. These three plants are often found in swamps, rivers, irrigation canals, and freshwater lakes, and can be used to remediate waters polluted by waste. Water lettuce has been found to have the potential to accumulate heavy metals, such as Ag, Cu, Ni, Pb, and Zn (Mursalin, 2007), while hydrilla can accumulate and absorb heavy metals with higher efficiency than *Salvinia molesta*, especially for copper (Cu) at a concentration of 5 ppm (Fuad, 2013). Similarly, the yellow bur head can thrive in polluted swamp areas due to its special adaptation pattern that allows it to survive in an environment that contains toxic elements or heavy metals (Widyati, 2011).

The use of plants as phytoremediator agents has been widely studied, but the results are incomplete. Nonetheless, studies on the abilities of *P. stratiotes, L. flava*, and *H. erticillate* to remediate heavy metals, such as Pb, Cd, Cr, and Hg, are not available. There has been no comparison of the growth of water lettuce, yellow bur head, and hydrilla when exposed to waste containing heavy metals as a reference for determining their potential as phytoremediator agents. Therefore, this study aims to provide comprehensive information on the ability of *P. stratiotes*, *L. flava*, and *H. verticillata* to remediate heavy metals, specifically Pb, Cd, Cr, and Hg.

Materials and Methods

This study spanned 8 months and, was preceded by a survey of the Arbes watershed's river conditions and the activities of the surrounding community that caused a decrease in water quality between May and June 2021. Following the survey, laboratory tests were conducted between July and December 2021 to analyse the B3 heavy metal content, specifically Pb, Cd, Cr, and Hg, in the water samples from the Arbes River, at the chemistry laboratory of Malang Muhammadiyah University, Indonesia. Additionally, the phytoremediation potential of *P. stratiotes, L. flava*, and *H. verticillata* was evaluated at the science laboratory of Ambon State Islamic Institute, Indonesia.

The river water sampling locations were determined using the "sample survey method" by dividing the study area into stations representing the population. Meanwhile, the sampling points were determined by dividing the river into six segments based on the characteristics of land use and community activities while considering ease of access, cost, and time to determine a segment that is representative of the water quality in the Arbes River.

The Arbes River is categorised as an intermittent river, where the water flow depends on the season. The water flow is quite large during rainy seasons and relatively small in dry seasons. Based on the water source, the Arbes River is an intermittent spring-fed river with the source coming from groundwater or springs in the upstream area. The study was conducted along the Arbes River, which has a length of approximately 9,832 km, from its upper stream in the Arbes Mountains to the Ambon State Islamic Institute Housing Complex. The observation was carried out by dividing the river into 6 segments based on the characteristics of land use and community activities.

The river water sampling locations started from segments 1 (upstream) to 6 (downstream). The coordinates of the sampling segments were 3^{0} 41’ 33.126” south latitude and 128^{0} 13’ 30,0504” east longitude to 3^{0} 40’ 50.8476” south latitude and 128^{0} 13’ 22.6812” east longitude. The sampling locations are described as follows:

Segment 1

Water sampling in segment 1 was carried out in the Arbes Mountains (Neighborhood 06, Hamlet 17) at coordinates 3^{0} 41’ 33.126” south latitude and 128^{0} 13’ 30,0504” east longitude. This area is the upstream part of the river, which is mostly used by the community for domestic purposes and partly for agriculture.

Segment 2

Water sampling in segment 2 was carried out in Arbes (Neighborhood 05, Hamlet 17) at coordinates 3^{0}41’25,1412” south latitude and 128^{0} 13’ 22,998” east longitude. This area is widely used by the community for domestic purposes.

Segment 3

Water sampling in segment 3 was carried out in Kehena (Neighborhood 10, Hamlet 17) at coordinates 3^{0} 41’ 8.9592” south latitude and 128^{0} 13’ 27.1956” east longitude. The general consumption of water in this area is also for domestic purposes.

Segment 4

Water sampling in segment 4 was carried out at the Kali Jodoh Bridge (Neighborhood 03, Hamlet 17) at coordinates 3^{0} 41’ 1.5612” south latitude, and 128^{0} 13’ 30.8028” east longitude. The surrounding community uses the water in this area mainly for domestic purposes.

Segment 5

Water sampling in segment 5 was carried out at the State Islamic High School Bridge (Neighborhood 02, Hamlet 17) at coordinates 3^{0} 40’ 58.0476” south latitude and 128^{0} 13’ 26,7168” east longitude. The water in this area is generally utilised for domestic purposes.

Segment 6

Water sampling in segment 6 was carried out at the State Islamic Institute housing complex (Neighborhood 01, Hamlet 17) at coordinates 3^{0} 40’ 50.8476” south latitude and 128^{0} 13’ 22.6812” east longitude. This area is widely used by the community for domestic purposes. The study locations in general, are shown in Figure 1.

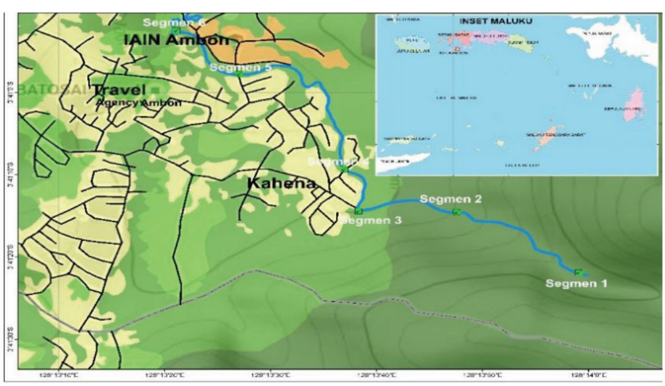

Figure 1: A map of the research locations: Segments 1, 2, 3, 4, 5, and 6

The river water sampling was carried out at a depth that is half of the river's total depth. This point was considered to be representative of the river's water quality because it is marked by perfect mixing or homogeneous flow. The sampling was conducted using a long-stemmed plastic scoop as per SNI 6989.59-2008 guidelines for wastewater sampling. Two polypropylene plastic containers with a volume of 5 litres were used to collect the samples, and the tests carried out on the samples are shown in Table 1.

Heavy Metal Remediation by Aquatic Plants

The phytoremediator agents used were *P. stratiotes, L. flava*, and *H. verticillata*. The three plants were acclimatised for 2 weeks in the laboratory before being treated. The plants were then left exposed for 4 weeks, and the final heavy metal content measurement was carried out in the growing media. The work steps carried out are as follows:

(a) An aquarium measuring 40 cm x 10 cm x 30 cm was used as a container to grow phytoremediator agents in waste from the Arbes River.

(b) Each container was filled with 5 litres of water from the Arbes River, containing known initial concentrations of lead, cadmium, chromium, and mercury heavy metals. Six phytoremediator plans were grown in each container.

(c) The parameters measured include the initial heavy metal content of lead, cadmium, chromium, and mercury and the heavy mental content after 1 month of treatment with the phytoremediator agents.

The data analyse involves assessing the quality of the Arbes River in terms of its B3 heavy metal content, including Pb, Cd, Cr, and Hg. In addition, the ability of three aquatic plants, namely *P. stratiotes, L. flava*, and *H. verticillate*, to remediate Arbes River water contaminated with B3 heavy metals were analysed descriptively.

Results and Discussion

Arbes River Water Quality

Water quality is the state that reflects the composition of living organisms, substances, energy, or other components in water. This state is characterized by parameters that describe the condition of the water, such as physical, chemical, and biological factors. These parameters are measured using specific methods in accordance with applicable laws and regulations. To determine the quality of river water, standard parameters are compared with the Government Regulation Number 82 of 2001 on Water Quality Management and Water Pollution Control. The data on water quality obtained from sampling and analyzed at the Chemistry laboratory of Malang Muhammadiyah University are presented below.

Lead Heavy Metal Content in water in the Arbes River

Table 2 shows the results of the Pb content observation and measurement in Arbes River across segments 1 to 6. The measurements indicate that the lead content from segments 1 to 2 is relatively low, at 0.03 mg/l, meeting the criteria for class I river water quality. Meanwhile, the Pb content in segments 3 to 6 is very high, exceeding the criteria for class III at > 0.03 mg/l.

Table 1: Measurement Parameters on Water Snippets from 6 Segments (Sampling Points)

No	Parameters	Required volume (ml)	Unit	Method	Treatment
1	Heavy metals (Pb, Cd, Hg, and Cr)	1000	mg/l	Spectrophotometry	Laboratory

Source: SNI 6989.59-2008 on the waste-water sampling method

Table 2: The results of Pb content analysis in Arbes River water

No.	Location	Pb (mg/l)	Water quality criteria, class (Government Regulation Number 82 of 2001)				Description
			I	II	III	IV	
1.	Segment 1	0.029	0.03	0.03	0.03	1	Fulfilling class I
2.	Segment 2	0.030					Fulfilling class I
3.	Segment 3	0.081					Exceeding class III
4.	Segment 4	0.082					Exceeding class III
5.	Segment 5	0.115					Exceeding class III
6.	Segment 6	0.114					Exceeding class III

Source: Primary data, 2021

Cadmium Heavy Metal Content in water in the Arbes River

The results of Cd content observation and measurement in Arbes River water from segments 1 to 6 are presented in Table 3. The results indicate that the Cd content in all segments is relatively high at > 0.01 mg/l and does not fulfil the class I river water quality criteria. The lowest Cd content was found in segment 1 (0.022 mg/l), while the highest Cd concentration was detected in segment 5 (0.117 mg/l) y.

Chromium Heavy Metal Content in water in the Arbes River

The results of Cr content observation and measurement in Arbes River water from segments 1 to 6 are shown in Table 4. The results show that the Cr content in segments 1 and 2 is relatively low at < 0.05 mg/l and fulfils the class I river water quality criteria. However, the Cr content in segments 3 to 6 is very high at > 0.05 mg/l and exceeds the class III quality criteria, indicating the water is not suitable for drinking but can be used for irrigation purposes.

Mercury Heavy Metal Content in water in the Arbes River

The results of Hg content observation and measurement in Arbes River water from segments 1 to 6 are shown in Table 5. The results show that the Hg content in segments 1 and 2 is relatively low at = 0.01 mg/l, but very high at = 0.005 mg/l in segments 3 to 6. The Hg content in segments 1 and 2 is within

Table 3: The results of Cd content analysis in Arbes River water

No.	Location	Cd (mg/l)	Water quality criteria, class (Government Regulation Number 82 of 2001)				Description
			I	II	III	IV	
1	Segment 1	0.022	0.01	0.01	0.01	0,01	Fulfilling class IV
2	Segment 2	0.023					Fulfilling class IV
3	Segment 3	0.083					Fulfilling class IV
4	Segment 4	0.084					Fulfilling class IV
5	Segment 5	0.117					Fulfilling class IV
6	Segment 6	0.115					Fulfilling class IV

Source: Primary data, 2021

Table 4: The Results of Cr content analysis in Arbes River water

No.	Location	Cr (mg/l)	Water quality criteria, class (Government Regulation Number 82 of 2001)				Description
			I	II	III	IV	
1	Segment 1	0.031	0.05	0.05	0.05	1	Fulfilling class I
2	Segment 2	0.033					Fulfilling class I
3	Segment 3	0.153					Exceeding class III
4	Segment 4	0.154					Exceeding class III
5	Segment 5	0.219					Exceeding class III
6	Segment 6	0.217					Exceeding class III

Source: Primary data, 2021

Table 5: The results of Hg content analysis in Arbes River water

No.	Location	Hg (mg/l)	Water quality criteria, Class (Government Regulation Number 82 of 2001)				Description
			I	II	III	IV	
1	Segment 1	0.001	0.001	0.002	0.002	0,005	Fulfilling class I
2	Segment 2	0.001					Fulfilling class I
3	Segment 3	0.005					Fulfilling class IV
4	Segment 4	0.005					Fulfilling class IV
5	Segment 5	0.007					Fulfilling class IV
6	Segment 6	0.007					Fulfilling class IV

Source: Primary data, 2021

the tolerance threshold, but in segment 3 to 6, it is at a threshold that is harmful to the survival of organisms.

The Pb content in Arbes River water in segments 1 to 2 was relatively low at 0.03 mg/l, meeting the class I river water quality criteria. However, the Pb content in segments 3 to 6 was very high at > 0.03 mg/l, exceeding the class III criteria. Meanwhile, the Cd content in segments 1 to 6 was quite high at > 0.01 mg/l, failing to meet the class I river water quality criteria. It is worth noting that cadmium is typically found in dissolved and suspended forms, and its solubility is relatively low, resulting in small amounts in water. Segment 1 had the lowest cadmium content at 0.022 mg/l, while segment 5 had the highest at 0.117 mg/l. The (Hg content in segments 1 to 2 was quite low at = 0.01 mg/l, but very high at > 0.005 mg/l in segments 3 to 6. It should be noted that the level of heavy metals in segments 1 and 2 is within an acceptable range, but in segments 3 to 6, it exceeds the threshold that can be harmful to the survival of organisms. Furthermore, the Cr content from segments 1 to 2 was quite low at < 0.05 mg/l, and this fulfils the class I river water quality criteria. However, the Cr content in segments 3 to 6 was very high, measuring at > 0.05 mg/l, and exceeds the criteria for class III criteria. While this level is not safe for drinking, it is still suitable for tourism and irrigation purposes.

The results show that the Arbes River water contains Pb, Cd, Cr, and Hg with varying concentrations. The presence of these heavy metals is caused by community activities, such as the disposal of domestic waste. In addition, Mount Arbes had seen illegal gold mining activities, which might still be ongoing without the government's knowledge. Furthermore, segment 2 is home to a hydroelectric power plant that has the potential to discharge waste into the river.

Heavy metals are natural components of the environment that need more attention from the community and the government due to their accumulative nature and harmful effects on ecosystems and human health (Khan *et al.,* 2015; Rai, 2019; Tauqeer *et al.,* 2021). The hazards associated with heavy metals have recently emerged as a significant environmental concern. Various hazardous wastes generated by human activities contribute to this issue, causing several problems. Although nature has a mechanism to mitigate the negative effects of heavy metal accumulation on the ecosystem, the quantities often exceed nature's processing capacity (Nejad *et al.,* 2018; Gupta *et al.,* 2019).

Water is often polluted by inorganic components, including dangerous heavy metals (Calheiros *et al.,* 2008; Ahmed *et al.,* 2016). Some of these heavy metals are widely used in various daily needs, which can directly or indirectly pollute the environment and be harmful to life when the content exceeds the specified limit. Dangerous heavy metals that often pollute the environment include Hg, Pb, Cd, and chromium (Li *et al.,* 2010; Rafati *et al.,* 2019).

Naturally, the metal rotation cycle begins from the earth's crust to the soil layer, then to living things, including plants, animals, and humans, and into the water, where they settle before finally returning to the earth's crust (Das *et al.,* 2016; Emenike *et al.,* 2018; Elbehiry *et al.,* 2020). The passage process of pollutants after entering the water is influenced by several factors or pathways. The findings are consistent with Romimohtarto (1991), where the possibilities for the passage of the contaminants include being scattered and spread out by mixtures, turbulence, or ocean currents, and concentrated through (1) biological processes of absorption by fish, plankton, marine algae, which are then consumed by other organisms; and, (2) physical and chemical processes through absorption, deposition and ion exchange, which cause them to settle at the bottom of the water bodies. Experts' opinions on the presence of heavy metals in the waters are consistent with the survey and interview results of the surrounding community's activities, such as disposing of domestic, livestock, human waste, and washing. These activities cause the river to experience a decrease in quality, which is indicated by the discovery of heavy metals, such as Pb, Cd, Cr, and Hg, with varying concentrations, thereby making the water unfit for drinking.

Phytoremediation Activity by Aquatic Plants

The ability of *P. stratiotes, L. flava,* and *H. verticillata* used to remediate B3 heavy metals, including Pb, Cd, Cr, and Hg, in Arbes River water is shown in Figure 2.

Figure 2 shows that the phytoremediator abilities of the three plant species in reducing the heavy metal content (Pb, Cd, Cr, and Hg) in Arbes River water differed. The initial lead content (0.115, exceeding the water quality standard's class III) decreased to 0.024 ppm, 0.017 ppm, and 0.009 ppm (class I), respectively, after administration (four weeks) of *P. stratiotes, L. flava*, and *H. verticillata*. On the other hand, Cd (0.117 ppm in class IV) decreased to 0.053 ppm, 0.042 ppm and 0.021 ppm in class II after the administration of the three plants. The Cr (0.219 ppm exceeded class III) was decreased to 0.062 ppm, 0.041 ppm and 0.040 ppm in class I after the administration of *P. stratiotes*, *L. flava,* and *H. verticillata*. Meanwhile, the initial Hg content was 0.007, exceeding class IV, but decreased to 0.003 ppm, 0.002 ppm and 0.0009 ppm (class I) after the administration of the three plants.

H. verticillata had a better ability to remediate lead and cadmium significantly higher than *L. flava* and *P. stratiotes.* However, the ability of *H. verticillata* and *L. flava* in remediating chromium is not significantly different,but *P. stratiotes* indicated an ominous

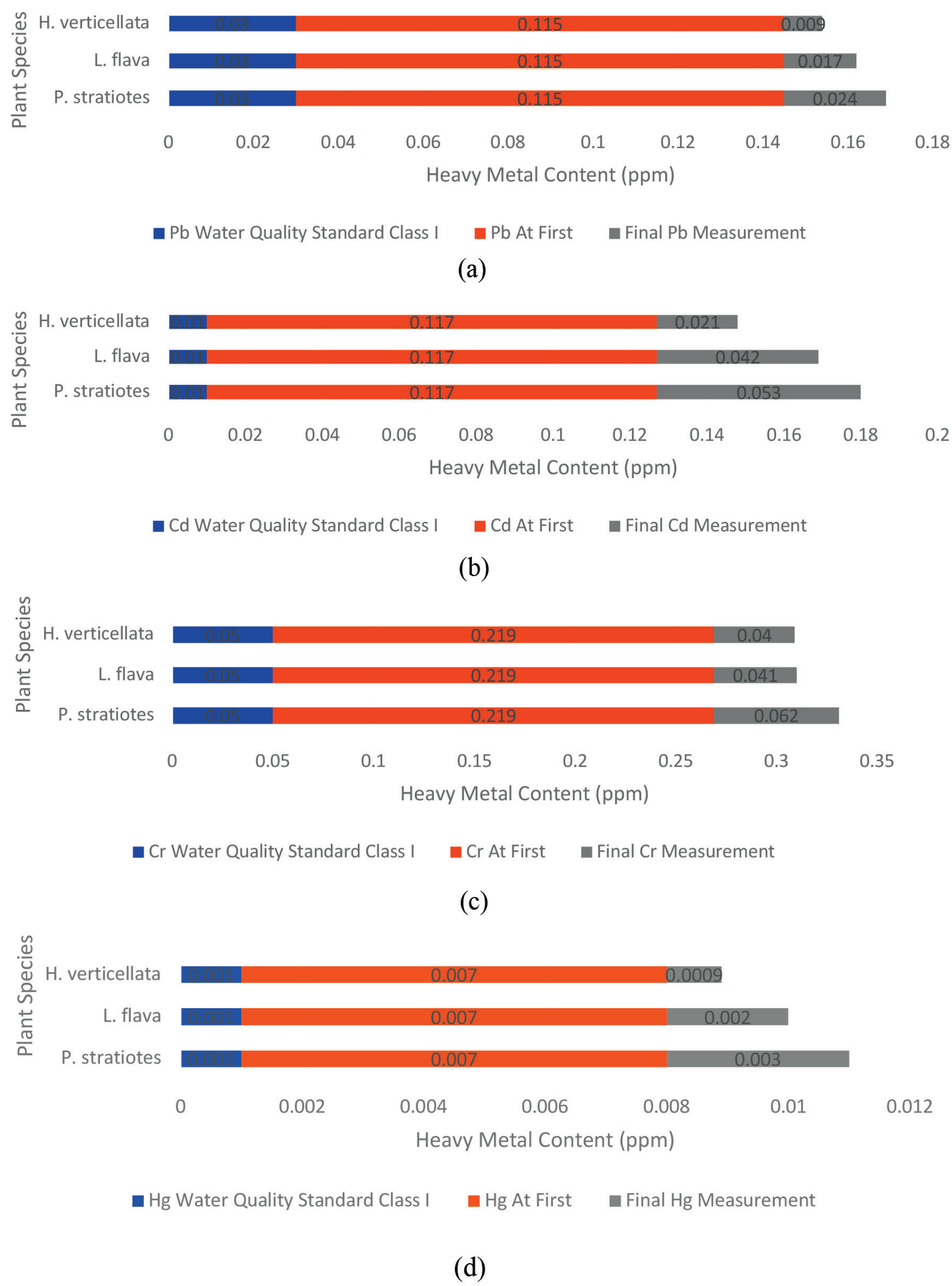

Figure 2. The ability of plants to remediate heavy metals: (a) Pb, (b) Cd, (c) Cr, and (d) Hg

result. The remediating ability towards mercury for the three-plant species was significantly different.

Three plant species, *P. stratiotes, L. flava,* and *H. verticillate*, were tested with waste from the Arbes River showed different abilities in reducing Pb. The results showed that *H. verticillata* had a better ability compared with *L. flava,* and *Pistia stratiotes*. Aside from Pb, *P. stratiotes, L. flava,* and *H. verticillata* tend to also reduce Cd. The results showed that the three phytoremediator agents had different abilities in reducing Cd, with *H. verticillata* having a better ability than *L. flava,* and *P. stratiotes*. The three-plant species reduced Cr after exposure for 4 weeks, but *H. verticillata* had a better ability than the other plants.

The three plants used as phytoremediator agents also can remediate Hg in Arbes River water. Based on the results, the three plants had different abilities, with *H. verticillata* having a better effect compared with *L. flava* and *P. stratiotes*.

According to Abou Seeda *et al.* (2020) and Babu *et al.* (2021), an important process in phytoremediation is rhizome-filtration, which is the deposition of contaminants, such as heavy metals by roots with the help of chelating agents. This is in line with Galal *et al.* (2018) and Haq *et al.* (2020), which reported that plants have certain mechanisms to prevent metal from poisoning the cells. An example is the accumulation of metals in certain organs, such as the roots. Furthermore, Farraji *et al.* (2020) and Shikha and Singh (2021) stated that plant species that grow in metal-polluted environments will experience metal stress by forming phytochelatins, especially in the roots, as an important tolerance mechanism. Phytochelatins are small peptides rich in the sulfur-containing amino acid cysteine (Padmavathiamma & Li, 2007; Chaney & Baklanov, 2017; Ma *et al.*, 2020).

The sulfur atoms in this system bind to heavy metals from the growing medium. Phytochelatin compounds found in plant roots bind metal elements and transfer them into cells through a passive transport process (Aruliah *et al.,* 2019; Liang *et al.*, 2017). Ulfin and Widya (2005) found that this absorption is the result of the diffusion law, namely the movement of Cr metal ions from a concentrated medium to a more dilute concentration, namely in plant membranes. Plants can accumulate these heavy metals by converting them into a liquid phase through cell metabolism with different stages.

The mechanism of plants in dealing with heavy metals can be accomplished in various ways, depending on the type of plant, namely through phytoextraction and phytochelatin (Chand *et al.,* 2015; Yan *et al.,* 2020). Phytoextraction is a form of phytoremediation in which plants, through the roots, absorb pollutants, such as heavy metals, from solutions and accumulate in stems and leaves which are harvestable plant parts (Cristaldi *et al.*, 2017; Galal, 2018). This mechanism is commonly used to recover polluted soil, especially heavy metals such as Pb (Fritioff & Greger, 2003). Plants with the ability to absorb heavy metals in higher amounts are called hyperaccumulators. They can accumulate heavy metals in plant tissues and harvestable parts in the range of 0.1% to 1% of their dry weight (Lasat, 2002; Chen *et al.,* 2015). Hyperaccumulation is a combination of adsorption, transport, and translocation aspects that require a large reservoir in the form of a storage or a tissue section to store pollutants or heavy metals.

This process mainly depends on heavy metals and plant species. The heavy metals absorbed by plant roots along with other nutrients are transported through the xylem and phloem tissues and then accumulated in the harvestable parts (Lasat, 2002; Garcia *et al.,* 2013). The adsorption of heavy metal pollutants by plants combined the advantages of a larger root surface area with a high affinity for chemical receptors. Within the root cells, transport systems and sites with high binding affinity mediate heavy metal uptake through the plasma membrane. Heavy metal uptake occurs through secondary carriers, such as channel proteins or H+ carrier proteins, while the negative membrane potential promotes cation uptake through secondary transport (Taiz

& Zieger, 2002; Lorestani *et al.*, 2013; Marrugo-Madris *et al.*, 2021).

The sequence of heavy metal uptake into root symplasm and movement to the xylem includes three stages, namely heavy metal retention in root cells, symplastic transport to stele, and, finally, release to xylem, which is mediated by membrane transport proteins. In the transport and translocation of heavy metals, phytochelatins and metallothioneins play an important role (Lasat, 2002; Padmavathiamma & Li, 2007; Jalali & Imanifard, 2021). Phytochelatin is a group of proteins containing the amino acids cysteine, glycine, and glutamic acid, which induce plants when they are under heavy metal stress. These compounds bind metal ions and transfer to vacuoles where heavy metals are no longer toxic. The mechanism of action for metallothionein is not very clear, but there are two hypotheses proposed. The first theory states that metallothionein creates an ion storage pool for excess chelated free heavy metal ions until plants use them when essential. The second theory states that metallothionein is a transport protein that is responsible for the transfer of excess heavy metals from a site, where it binds to toxic contents to sites where heavy metals are needed (Taiz & Zieger, 2002; Newete & Byrne, 2016; Farraji *et al.,* 2020; Tauqeer *et al.*, 2021).

Phytochelatin is a protein produced by plants as adaptation exposure to heavy metal content environments (Emenike *et al.,* 2018; Haq *et al.,* 2020). Vatamaniuk *et al.* (2000), mentioned that phytochelatins are small peptides rich in sulfur-containing amino acids. They usually have two to eight units of cysteine at the centre of the molecule, with glutamic acid and glycine at opposite ends. Proteins are very complex compounds that always contain the elements carbon, hydrogen, oxygen, nitrogen, and often sulfur. It is composed of amino acid molecules that are linked together to form long chains. This relationship occurs by combining the carboxyl group of one amino acid with the amino group of another acid, along with the removal of one molecule of water from the compound, namely a condensation reaction. The bond – CO – NH – that joins the two amino acids is called a peptide bond. Cysteine is an example of an amino acid that contains sulfur, besides the four primary elements. This acid deserves special attention because the sulfhydryl group, –SH, is very reactive and on oxidation, combines with the sulfhydryl group of another cysteine molecule to form a double amino acid.

According to Salisbury and Ross (1995), phytochelatins are produced by several plant species. However, based on previous studies, they are only found when there are toxic amounts of metals present. Therefore, it is formed in a plant as a response to adapt to a vulnerable environmental condition. Vatamaniuk *et al.* (2000) showed that the binding of metals to phytochelatins led to the formation of metal complexes that detoxify to make plants capable of withstanding heavy metal stress. According to the results, the ability of *H. verticillata* to remediate Pb, Cd, Cr, and Hg was greater than that of *P. stratiotes* and *L. flava*. This is because all body parts of *H. verticillata* are immersed in water, hence, the entire body surface has a high possibility of absorbing heavy metals.

The results also showed that *L. flava* did not grow in an aquarium containing wastewater from the Arbes River, while *P. stratiotes* and *H. verticillata* grew adequately as indicated by the increase in height during 4 weeks of exposure to wastes. The average height increase of *P. stratiotes* was 2.07 cm and 9.99 cm for *H. verticillata*. Moreover, the root length is a very important growth indicator to be measured and is related to the mechanism of heavy metals and coliform absorption. The results showed that *L. flava* did not grow in an aquarium containing waste, while *P. stratiotes* and *H. verticillata* grew adequately as indicated by the increase in root length during 4 weeks of exposure. The average increase in root length of *P. stratiotes* was 5.55 cm and 3.65 cm for *H. verticillata*. Another growth parameter was wet weight after 4 weeks of exposure. The results showed that *P. stratiotes* and *H. verticillata* had poor growth. The average wet weight increase of *P. stratiotes* was 6.81 g and 2.94 g for *H. verticillata*.

Based on the growth parameters observed in the three aquatic plants used to remediate B3 heavy metals, namely Pb, Cd, Cr, and Hg, hydrilla had better growth in terms of height and root length than water lettuce and yellow bur head. However, the wet weight of water lettuce was higher than hydrilla and yellow bur head. For the increase in height and root length, hydrilla had better results than the other two plants as it can adapt to polluted environmental conditions. The three plants showed great potential in remediating waste containing heavy metals and coliforms. However, in terms of the ability to grow in polluted environments, hydrilla and water lettuce are very tolerant as indicated by the increase in height, root length, and wet weight during 4 weeks of waste exposure.

Metal-tolerant plant species have defence mechanisms related to cellular antioxidants and enzymes that protect several vital physiological processes to prevent damage caused by reactive forms of oxygen due to stress caused by metal content (Panda & Choudhury, 2005; Chaney & Baklanov, 2017; Farid *et al.,* 2017; Yilmaz & Kokten, 2021). Tolerance or resistance of plants to metal stress is often associated with one or more mechanisms (Patra, 2004), such as (1) chelate excretion; (2) metal excretion through absorption of certain elements; (3) metal storage in roots, which prevents metal translocation to aerial parts; (4) sequestration of heavy metals by ligands, compartmentalisation, biotransformation, and cell repair mechanisms; (5) development of metal-tolerant enzymes; (6) increased production of intracellular compounds; (7) metal immobilisation in the cell wall; (8) homeostatic cell mechanism to regulate metal ions in the cell; (9) induction of heat-shock protein; (10) release of phenol from roots; (11) increased tolerance to mineral deficiency or decreased nutrient requirements; (12) increased absorption of certain macronutrients; and, (13) development of the capacity to continue absorbing and using minerals despite the presence of heavy metals in the plant.

Conclusion

The water quality of the Arbes River is based on measurements of B3 heavy metals, namely Pb, Cr, Cd, and Hg, in six segments. On average, it exceeds class III and class IV, which means that the water is not suitable for drinking, but can be used for irrigation. However, the use of aquatic plants can be a solution in remediating the heavy metal content. *H. verticillata* was found to have produced a better remediation effect on Pb, Cd, Cr, and Hg content than *L. flava* and *P. stratiotes.* Therefore, it is a potential phytoremediator agent in remediating heavy metals in the Arbes River. It is recommended that the community living around the river reduce activities that pollute it, such as washing, bathing, and disposing of domestic waste. Besides, the use of *P. stratiotes, L. flava,* and *H. verticillata* is suggested for cleaning river water contaminated by B3 heavy metals using the floating raft technique.

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