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FLOATING PLANT CAPACITY *Azolla Pinnata* var. *pinnata (A. pinnata)* TO TREAT HEAVY METALS Cr, Cu, Mn and Zn IN SURFACE WATER: CASE OF ANDRALANITRA

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ABSTRACT

The human health and the ecosystem face a serious problem day by day because of the rising of minerals pollutants especially heavy metals in the environment. Thereby, human is seeking processes and adopts to treat the places that he has polluted, though most of the time, those techniques are expensive and complicate at its implantation, its manipulation or its maintenance, but the most serious is that the treatment of pollutant causes another pollutant. The use of a simple technique, efficient, economically competitive and able to preserve the specifications of aquatic environment will be important then. The use of biotechnologies has proved to be an interesting alternative; this is where phytoremediation comes in. This study aims to evaluate the capacity of the aquatic plants Azolla pinnata var. pinnata (A. pinnata) from Azollaceae family to treat heavy metals Cr, Cu, Mn and Zn in the wastewater: case of Andralanitra.

Around 500g of the plant have been put into a reactor then planted in the surface water of Andralanitra. During the experimental study, around 50g of plants and 1L of water are gathered every day for the analysis at the laboratory. The level of metals in the Andralanitra's water is respectively 70.86 µg Cr.L^{-1}; 121.52 µg Cu.L^{-1} 265.71 µg Mn.L^{-1} and 132.55 µg Zn.L^{-1}. The plant performance to treat the Cr is at 1255% on the fourth day of treatment, the Cu is at 2027% on the seventh day and the Zn is at 417,1% on the third day. Finally, A.pinnata accumulates 151.8 mg Cr.kg^{-1} in its dry weight; 683.1 mg Cu.kg^{-1}; 1161.9 mg Mn.kg^{-1} and 672.3 mg Zn.kg^{-1}. Thereby, the results of the study show us that the aquatic plant A. pinnata has a strong potentiality to treat effluents containing the heavy metals Cr, Cu, Mn and Zn.

KEYWORDS: ***Andralanitra, Azolla pinnata, Chromium, Copper, Manganese, Phytoremediation, Wastewater, Zinc,***

1. INTRODUCTION

The advancement of current technology required an important and ongoing resource of basics materials. With that evolution, human needs for mining products keep increasing and they will be forced to increase the production. The extraction and the use of those mining products in industrial processes are the main sources of the problem for the environment, especially on environment pollution by heavy metals (Godin, 1983; Wu et *al*, 2015). Human health and ecosystem face a great danger gradually the fact of the accumulation of those minerals pollutants in the environment (Goix et *al*., 2015; Lévêque et *al*., 2015; Austruy et *al*., 2016), not to mention the inherent toxicity of heavy metals, they tends to accumulate and to persist in the ecosystem.

Many of those heavy metals are micronutrients essential for the plant development and growth and living organisms, but at a high dose, they become toxic and destroy the life of the organism (Wilkins, 1978, Tu and Broutlettic, 1987; Vazquez et *al*., 1987). However, those which are not essential for organisms are toxic even at low concentration, like the mercury (Hg), le cadmium (Cd), le plumb (Pb) and the arsenic (As) (Bennicelli et *al*., 2004; Marschner, 1995).

In the face of this rapid evolution of industrialization, it keeps releasing a large amount of heavy metals in the environment; therefore, the natural self-purification process which exists in the space will no longer be able to perform its role. Thereby, men are in search of methods to treat the environments they have polluted, though, in most of the cases, those techniques are expensive and more complex in term of its implantation and its manipulation or its maintenance (Godin, 1983), also and the most serious is that the treatment of a pollutant leads to another, this is because of products or materials, either by devices used. It is important to practice a simple, effective technique that can preserve the characteristics of the aquatic environment, also a less expensive technique than the techniques which are already used. The use of biotechnology has proved to be an interesting alternative; this is where phytoremediation comes in (Andreas and Heinz, 2005; Thi, 2009; Vishnoi and Srivastava, 2008). Many aquatic plants have great capacity to accumulate the toxic pollutants by their different mecanisms and can purify aquatic environments highly polluted by industrial effluents or by agrochemistry (Elizabeth, 2005; Obek, 2009). Experimental studies confirm that *A. filiculoides* is Pb accumulator (Benaroya et *al*, 2004), *A.microphylla* Cd accumulator (Tan et *al*., 2011) and *A.caroliniana* Cr accumulator (Vimal, 2012). They are used to decontaminate or to reduce the pollutants rate in the aquatics environments (Brooks et *al*., 1977). To be able to evaluate the purification capacity of the plant *A. pinnata*, we will evaluate up to what quantity of pollutants, could it treat Cr, Cu, Mn and Zn metals in wastewater? In this experimental study, we will (1) Determine the physicochemical characteristics of the surface water of Andaralanitra and the well water used by the residents; (2) Measure the Cr, Cu, Mn and Zn daily quantity absorbed by *A. pinnata* according to physicochemicals, climatic and demographic conditions of the surface water of Andralanitra. (3) Calculate the Bio-concentration factor (BCF) and (4) Evaluate the heavy metals purifier capacity Cr, Cu, Mn and Zn of *A. pinnata*

2. MATERIALS AND METHODES

2.1. Preparation of samples

A. pinnata was identified in Flore department, Herbier Division of the Botanic and Zoological Park of Tsimbazaza in Antananarivo. It is harvested in the watercress at the bottom of the Fokontany of East Ampandrana, district of Antananarivo III, Antananarivo urban town. Harvested and sorted plants are washed many times in double-distilled water before being sown in SIS recommended by OCDE at the laboratory (Falihery, 2012; OCDE, 2006) for pre-culture. The duration of the pre-culture is at least 30 days. Stem plants from that pre-culture are used for our experimental study.

Around 500g of *A. pinnata* from preculture were used for the experimental study. The plant *A. pinnata* is introduced in a rector (Plastic bins surrounded by polystyrene and that the bottom is filled with small holes of around 2mm of diameter). During the 8-day duration of the experiments, 50g of plant samples in the bin was collected. The water samples were collected daily from well water and surface water and together with plant samples. Both the surface water and plant samplings were accomplished during eight days, but the 3 first days of the experimental study for the well water. The plant samples are sent directly to the laboratory, washed with tap water, rinsed twice with double-distilled water then dried in the oven at 60°C for 24h (Vimal, 2012). The heavy metals concentration from the plant samples was measured from the dry weight (DW).

2.2. Physical-chemistry analyses

The physicochemical characteristics including the pH, electrical conductivity (EC) and temperature of the surface water and the well water are measured with a multimeter numeric CONSORT C535, in-situ. The analysis are carried out by electrometry. To determine the characteristics of the surface water and the well water, 2 types of analyses were done: the first in-situ and the second at the laboratory. For the laboratory analyze, a sample was sent at the laboratory to determine the concentration of metals as well as the other remaining parameters. Different heavy metal concentrations were analyzed by atomic absorption spectrophotometer AAS VARIAN 240 FS (Rodier, 2009).

In this experimental study, the field culture of *A. pinnata* is done after the rainy season, i.e. from May. The absorption rate of metals by the plant can be valued by the determination of the variation of the concentration in terms of time and it is valued through the link between the absorbed concentrations with the treatment duration. Bioconcentration factor (BCF) is the ratio of heavy metal accumulated by plants to that dissolved in the aquatic medium (Dunbabin and Bowmer, 1992; Jitar et *al*., 2015). That parameter was calculated by the relation:

$$\mathbf{BCF= C_p/ C_s}$$

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C_p: *Heavy metal concentration in the biomass (mg.kg⁻¹)*

C_s: *Heavy metal concentration in water samples (mg.L⁻¹)*

2.3. Statistical analysis

All data were treated by Microsoft office Excel 2013 software and statistical analysis was carried out using Analysis of Variance (ANOVA) on Xlstat 2014.5.03. The differences were statistically significant when *p-value* was less than 0.05. However, the average of each result is compared with the average witness using the 95% confidence interval Dunnett's multiple comparison test. The correlation between the metal concentration in the surface water and the metal accumulates in the cellular tissues of the plant is analyzed by using Pearson's correlation coefficient test ($p<0.05$) (*Table 3*).

2.4. The study area

At 12km to the east of the down-town, Andralanitra is in the rural commune of Ambohimangakely, district of Antananarivo Avaradrano. It is the solids waste dumping site of the capital of Madagascar (*fig. 1*). It spreads on 18 hectares and has about 2.5 millions of m^3 of waste (MEEH Malagasy, 2017). Any global and recurrent study on the characterization of waste has not been carried on and that garbage (household waste, hospital waste, industrial waste) does not undergo any sorting. Otherwise, the last official characterization of landfill waste was carried out in 2003 by the SAMVA (Maintenance Autonomous Service of Antananarivo). As that site is an open-pit landfill place, it is a large site of chemical pollutants. And even if the site is surrounded by brick wall, that does not prevent the dispersion of liquid, solids, gaseous pollutants and many other waste around the place.

Rubbish is washed-out by the rain and emits liquid waste. However, apart from the infiltration of lixiviate in the soil, much of it spills into a surface water which are around the site; they are transported by a pipeline made by the municipality (*figure 2 and 3*). They use the phenomenal of dispersion for the effluent treatment which are heavily discharge with pollutants mostly chemical pollutants. The flow of the lixiviate emission is not constant as well as the pollutants which are there, they vary according to the season and the type of thrown garbage; it is between October and April, rain season, that the lixiviate diffusion is maximal (Madagascar Artelia, 2014). The average annual precipitation in the region of Antananarivo is 1346 mm, with an average maximum in January sometimes reaching 295 mm (rain season) and an average minimum of 8mm in June (dry season) (Madagascar Artelia, 2014).

Fig. 1 : Garbage storage site of Andralanitra

Fig. 2 : Lixiviate exit following the pipeline

Fig. 3 : Discharge of leachate into surface water and rice fields

Fig. 4 : A. pinnata cultivation in Andralanitra surface water

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Fig. 5 : In-situ analysis of the physic-chemical parameter of Andralanitra surface water

In one side, lixiviates pour out in the water surface of Andralanitra. However, people use that contaminated water as water for their agricultural irrigation (market gardening, rice cultivation) and fish farming.

In another side, before the arrival of the fountain provided by JIRAMA (drinking water supplier of the country), people used well water for consumption and for daily use. When the water of JIRAMA arrives, people do not use the well water anymore except for the case of fountain supply disruption. In point of fact, water supply in that eastern part of Antananarivo is not currently ongoing. Citizen do not have access to drinking water for a maximum of one hour per week. In that case, almost 90% of the family who live in the Fokontany reuse the well water for their daily needs including the cooking and the drink (field survey). However, the toxic pollutants like heavy metals will accumulate in the sediments then infiltrate in the soil and contaminate the underground water (Laura et *al.*, 2014), thereby, those pollutants will get directly into human bodies through those water (Groupe scientifique sur l'eau, 2019).

3. RESULTATS AND DISCUSSION

3.1. Physicochemical characteristics of well water of Andralanitra

The mean of chemical analysis and physicochemical characteristics of well water in Andralanitra are given in *Table1.*

Table 1 : Physicochemical characteristics of well waters. Sampling is done every day for 3 consecutive day. The value represented are the average value ± standard deviation.

Paramètres	T (°C)	pH	CE (mS.cm^{-1})	NO_3^- (mg.l^{-1})	HCO_3^- (mg.l^{-1})	SO_4^{2-} (mg.l^{-1})	MES (mg.l^{-1})
DL[1]	-	-	-	-	-	-	-
Surface water	24,83 ± 0,76	5,88 ± 0,30	3,27 ± 0,18	23,35 ± 0,89	128,07 ± 2,65	121,26 ± 3,91	5,3
Normes[2]	20 à 25	6,0 à 8,5	< 0,25	< 50	-	< 250	< 30

Ca (mg.l^{-1})	Co (µg.l^{-1})	Mg- (mg.l^{-1})	Cu (µg.l^{-1})	Mn (µg.l^{-1})	Zn (µg.l^{-1})
0.05	0.08	0.05	0.1	0.05	0.5
#	40,09 ± 1,60	#	#	9487,83 ± 41,10	103,36 ± 2,30
-		-	2000	400	3000

#: Below the detection limit for SAA

[1] *Detection Limits of AAS*
[2] *Decree n° 2003/464, MME and WHO/SDE/WSH/03.04/88*

As shown in *Table1*, mean Cu, Zn and same anions concentrations in the well water are lower than the limit values recommended by the WHO (World Health Organization) and MME (Malagasy Ministry of Environment), Mn and some physicochemical parameters (EC, pH) are beyond the limit values. The Mn is the dominant metal in well water, followed by Zn, and the Co. The other metals are less than the detection limit of Atomic Absorption Spectroscopy (AAS). The Mn concentration average is around 9487.83 ± 41. 10 $\mu g.L^{-1}$ a rate well over 400 $\mu g.L^{-1}$ concentration recommended for drinking water. While that of zinc is 103.36 ± 2.30 $\mu g.L^{-1}$ (the limit dose is 3000 $\mu g.L^{-1}$(WHO, 2006)) and that of Co is 40.09 ± 1.60 $\mu g.L^{-1}$

3.2. Physicochemical characteristics of the surface water of Andralanitra

Table 2: Physicochemical characteristics of the surface water

Parameter	T (°C)	pH	CE ($mS.cm^{-1}$)	HCO_3^- (mg.l-1)	Cl^- ($mg.l^{-1}$)	Fe_T ($mg.l^{-1}$)	Cr ($\mu g.l^{-1}$)
LD	-	-	-	-	-	0.01	0.1
Surface water	26,62 ± 0,50	6,77 ± 0,52	24,89 ± 0,76	9542,75 ± 53,18	5630.63 ± 95,14	3,00 ± 0,12	70,86 ± 5,44
Standard	20 à 25	6,5 à 9,0	0,2	-	< 250	5,0	200

Al ($\mu g.l^{-1}$)	Mg ($mg.l^{-1}$)	Cu ($\mu g.l^{-1}$)	Na ($mg.l^{-1}$)	Ca ($mg.l^{-1}$)	Mn ($\mu g.l^{-1}$)	Zn ($\mu g.l^{-1}$)
0.1	0.05	0.1	0.05	0.05	0.05	0.5
136,52 ± 2,24	42,14 ± 0,05	121,52 ± 2,47	7475,5 ± 36,08	24,49 ± 0,09	265,71 ± 0,83	132,55 ± 1,62
5000	-	200	-	-	5000	500

The water sampling for analyze is done every day for 8 days of field cultivation. The first day of the culture is taken J_0. The Mg, the Ca and the Na are dominant cation at the Andralanitra's surface water. They represent around 98% of metals. While the bicarbonate and the chloride are the majority anions, they occupy till 95% of the site. It is obvious that the contamination of the surface water and the underground water by the heavy metals depend on the source those last and their geochemical mobility. The average value of Cr, Cu, Mn and Zn in surface water concentration are 70,86 ± 5,44 $\mu g.L^{-1}$, 121,52 ± 2,47 µg/L, 265,71 ± 0,83 µg/L and 132,55 ± 1,62 µg/L, those values and the concentration average of the other elements and the physicochemical characteristics pH, T (°C) and the electrical conductivity EC ($mS.cm^{-1}$) of the surface water area are given in *table 2*. The pH values of the place during the 7 days of culture vary between 6,17 and 7,54 (6,77 ± 0,52); that of EC are between 24,21 $mS.cm^{-1}$ and 26,12 $mS.cm^{-1}$ (24,89 ± 0,76 $mS.cm^{-1}$) and that of the temperature are between 25,50 °C and 28 °C (26,62 ± 0,50 °C) ($p< 0.05$). We find that the values of each parameters in the 8 samples are similar and the concentration of each element during the treatment does not undergo a great variation, a bit variation during the 8 days of culture. That is because of weak movement of the middle; which is rubbish lixiviates flow time to the surface water, the dispersion time and mineralization of pollutants from lixiviats (Merve *et al*., 2015) and the sediment stability.

3.3 Purifying capacity of Cr, Cu, Mn and Zn by A. pinnata

Table 3 : Pearson's correlation coefficient between the values of the concentration of metals absorbed in the A. pinnata dry weight (DW).

	Cr	Cu	Ca	Mn	Al	Mg	Zn	Fe
Cr	**1**							
Cu	0,5533	**1**						
Ca	0,4066	**0,8843**	**1**					
Mn	**0,7742**	**0,9112**	**0,8584**	**1**				
Al	0,3606	0,0580	0,3573	0,2438	**1**			
Mg	0,3597	0,1895	0,0669	0,2325	0,0831	**1**		
Zn	**0,8038**	0,4934	0,4368	**0,7068**	0,3677	0,1738	**1**	
Fe	**0,8657**	**0,8646**	**0,7254**	**0,9246**	0,2766	0,4427	0,6998	**1**

Values in bold are different from 0 at a significant level α = 0.05

Metals such as Cu, Zn, Ag, Hg, Cd, As, Cr, Tl, Mn and Pb are toxic because of their high density and they are dangerous because they have the ability to accumulate to a high dose in biological tissues (Baby *et al.*, 2010 ; Austruy et *al.*, 2016). At low dose, some among those metals are necessary for the survival for living organisms such as Human and plants and each of them has their biological role (Heller, 1974 ;Tremel-Schaub and Isabelle, 2005).While, only have toxic properties like the Pb, As or the Hg.

3.3.1. Accumulation of chromium by *A. pinnata*

The chromium amounts in *A. pinnata* before the experimental study is 11.2 mg.kg^{-1} of the dry weight (DW). That concentration can be accepted as control groups for the test with that metal.

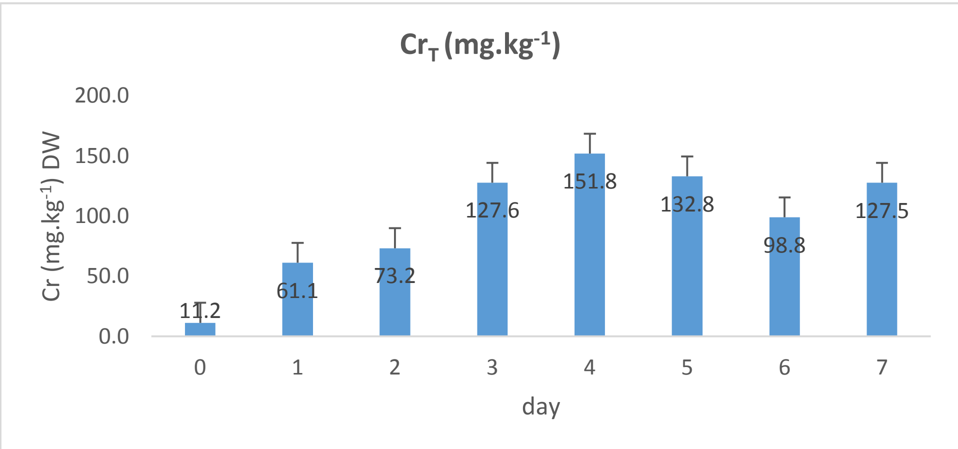

Fig.6 : Accumulation of chromium (mg.kg^{-1}) by A. pinnata

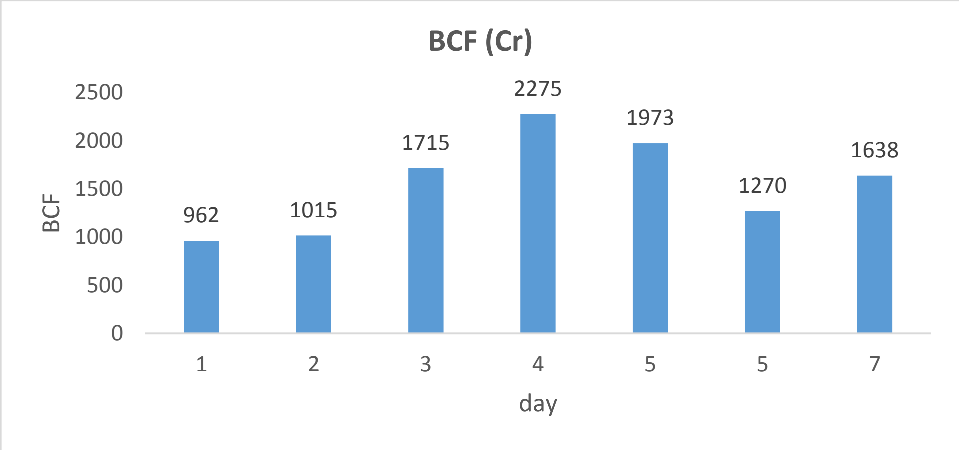

Fig. 7 : BCF A. pinnata of chromium metal

The different forms of chromium do not have the same effect or the same action sites. Organisms weakly absorb trivalent ions of chromium but hexavalent ions is more dangerous because its compounds easily penetrate through physiological barriers. The Cr (VI) pass though the cell membranes then transform into Cr (III) metabolite that can bind with protein. It is in this form that this element is more stable (Gauglhofer and Bianchi, 1991).
On the other hand, Cr (III) does not pass though the cell membrane thus it is only absorbed in small amounts (Behrouz, 1995). The *table 3* shows us that, that pollutant is a strong correlation with ions manganese, zinc and iron. The Pearson's correlation coefficient ($p<0.05$) are 0.7742 (Cr-Mn), 0.8038 (Cr-Zn) and 0.8657 (Cr-Fe), respectively.

The first day of the experimental study, *A. pinnata* accumulated 61.1 $mg.kg^{-1}$ (a performance of 445.5%). The plant can accumulates a quantity of chromium 1000 times greater than that found in the effluent, knowing that the chromium rate in the place is around 70.86 $\mu g.L^{-1}$ (*table 1*). The concentration of the accumulated chromium in the biomass increases during the 4 first days of the experimental study (*Fig. 6*) and we find that at this time, the chromium accumulation by *A. pinnata* is maximum. In that case, the chromium rate in the tissue is 151.8 $mg.kg^{-1}$. Thereby its performance is 1255%. The fifth day of the experimental study, the plant performance is 1085,7% or an accumulation of 132.8 mg $Cr.kg^{-1}$ DW and the sixth, the performance is 782.1% or an accumulation of 98.8 mg $Cr.kg^{-1}$ DW. The *fig.7* shows us the variation of BCF of the plant, its minimal value is obtained on the first day of the experimental study (BCF equal to 962), then again, that parameter is maximum on the fourth day of the survey, and it is equal to 2275. Comparing it to *A. caroliniana*. *A. pinnata* has a strong potential to process chromium because of its BCF 6 times greater. The BCF of that last one is equal to 349 (Vimal, 2012).

3.3.2. The accumulation of copper by *A. pinnata*

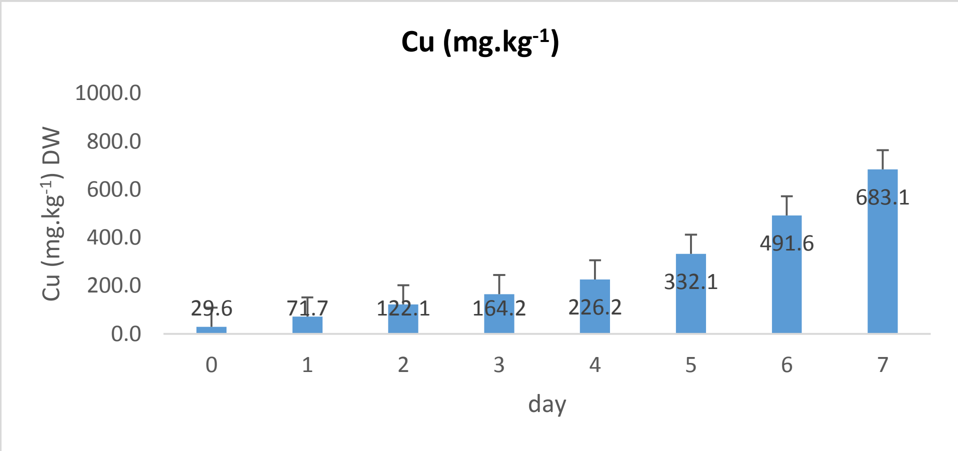

Fig. 8: Accumulation of Copper (mg.kg^{-1}) by A. pinnata

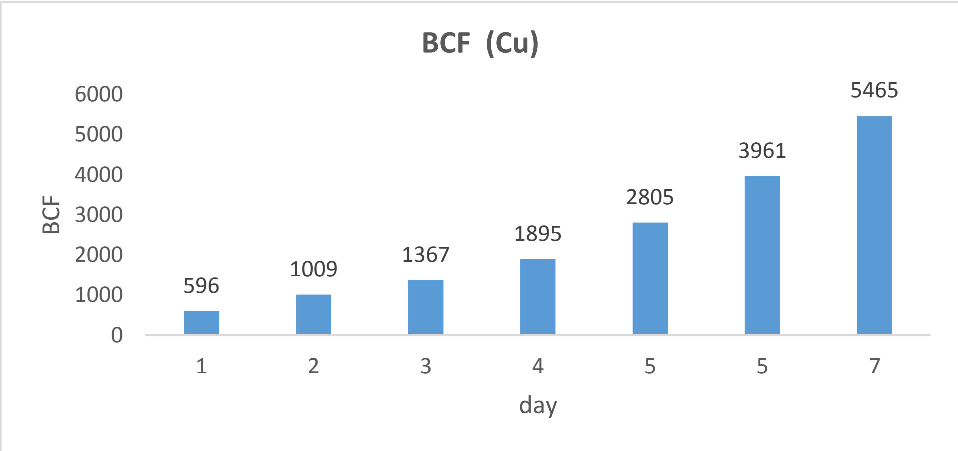

Fig. 9 : BCF A. pinnata of copper

The copper rate in the plant that used at the beginning of the experience is around 29.6 mg.kg^{-1}. Its content in the dry weight of the plant is around 20 ppm (Behrouz, 1995) thereby, the initial value found in *A. pinnata* is suitable and that concentration will be used as fiducial for the study with the other concentrations of copper. The copper is an essential element for the plant (Frank, 1952; Upadhyay et *al.,* 2007; William, 2003). It is a microelement for the plant metabolism, it has an important role in the cell structure and for the energy transfer to the plant. The copper ensures the stability of the protein and the chromosomes structures (Dawson and Mallette, 1945; Pahlsson, 1989; Upadhyay et *al.,* 2007).

In this study, the copper accumulation by *A. pinnata* increases till 142.23% after the first day of culture, it accumulated 71.7 mg.kg^{-1} in its tissue. The plant performance to accumulate the copper in its tissue increases during the testing. On the second day of the experimental study, the performance of *A. pinnata* to accumulate the copper is at 312.5% (122.1 mg Cu.kg^{-1}) and 454.7% on the third day (that is to say an accumulation of 164.2 mg Cu.kg^{-1}). The following days, the *A. pinnata* purifying power are respectively at 664.2%, 1022%, 1560.8% and 2207.8%. The *Fig. 8* shows us that the maximal absorption appears at the seventh day of the test, *A. pinnata* accumulated 683.1 mg Cu.kg^{-1}. On the other hand, the absorption is minimal on the first day of treatment, in that case it accumulated only 71.1 mg Cu.kg^{-1} DW. Also, the level of copper in the surface water shows a bit variation during the 7 days of experience and the BCF is maximal on the seventh day of experience. By comparing the value of this parameter to that of *A. caroliniana*, *A. pinnata* (BCF equal to 5465 after 7 days) has a strong potential on the treatment of the copper because its BCF is almost 275 times greater compared to *A. caroliniana*. The BCF of that *A. caroliniana* is around 20.4, a value obtained by Vimal, (2012).

Copper has a strong linear correlation with Ca, Fe and Mn ($p < 0.05$) (*table 3). *Ning et *al.*, (2011) observed in their study a strong linear correlation between Cu - Cd and Cu - Hg. The correlation

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coefficient of Cu-Cd is 0.980 while, that of Cu-Hg is 0,986. Merve et *al.,* (2015) have also found positives correlations between Cu-Ca (correlation coefficient 0.87) and the Cu-Mg (correlation coefficient 0.87) when treating a site with a high dose of heavy metals, with aquatic plants, on the other hand, in the same work, they confirmed negative correlation between the Mn (correlation coefficient -0.71) and Cu-P (correlation coefficient -0.86).

3.3.3. Accumulation of manganese by *A. pinnata*

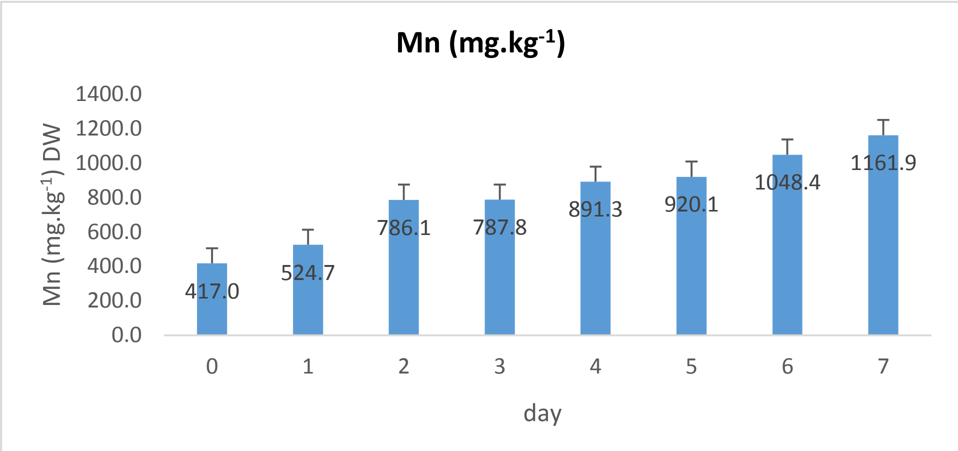
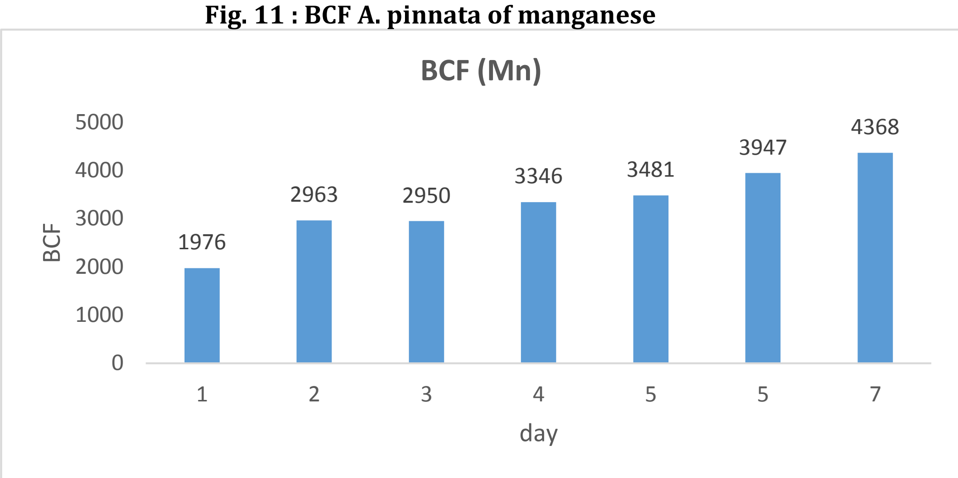


Fig. 10: Accumulation of Manganese (mg.kg^{-1}) by A. pinnata

Fig. 11 : BCF A. pinnata of manganese



The initial (j_0) of manganese amounts in *A. pinnata* is 417.0 mg.kg^{-1}. That values of manganese was accepted as control for *A. pinnata*, for the reason that the quantity of the metal in the dry weight plants varies between 10 and 1000 ppm (Behrouz, 1995). Thereby, we use that concentration as reference for the continuation of the experiment with manganese.

Mn accumulation of *A. pinnata* in the wastewater of Andralanitra stated increased during the experimental study. On the first day, the plant accumulated 524.7 mg Mn.kg^{-1}, which is a performance of 25.8% and 786.1 mg Mn.kg^{-1} on the second day, analogous to an accumulation capacity of 88.5%. On the third day of cultivation, *A. pinnata* accumulated 787.8 mg Mn.kg^{-1}, a slight difference compared to the second day but the plant capacity is 88.9% of reference. For the 4 last days of survey, the accumulations of manganese by *A. pinnata* are: 113.7% on the fourth day, then 120.6% on the fifth day, 151.4% on the sixth and 178% on the seventh day. Therefore, the accumulation of manganese by *A. pinnata* on the seventh day of the experimental study is maximal, we find 1161.9 mg Mn.kg^{-1} DW and the BCF is equal to 4368 (*Fig. 11*). Comparing its values to other species of Azolla: the BCF of *A. pinnata* is vastly superior to *A. caroliniana*'s BCF which is equal to 5.4 (Vimal, 2012) but slightly lower than that *Lemma minor* is equal to 5100 (Jamnicka et *al.,* 2006). Thereby, we can use *A. pinnata* to treat the manganese. The production of biomass is very fast and it can accumulate a great quantity of pollutant. The *table 3* shows us the correlation coefficient of Person between Mn-Cu (0.912); between Mn-Ca (0.8584); between Mn-Cr (0.7742); between Mn-Fe (0.9246) and between Mn-Zn equal to 0.7068 ($p<0.05$). Therefore, there is a strong linear correlation between Mn-Cu, Mn-Ca, Mn-Cr, Mn-Fe and Mn-Zn.

3.3.4. Accumulation of zinc by *A. pinnata*

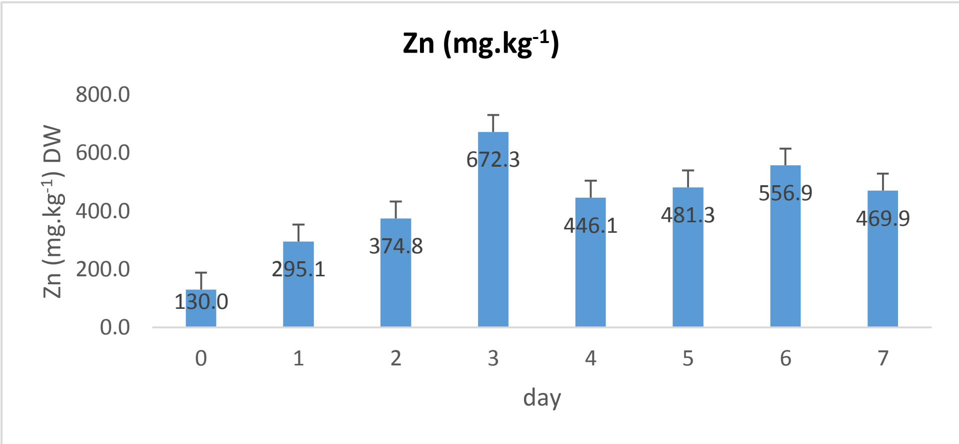


Fig. 12: Accumulation of Zinc (mg.kg^{-1}) by A. pinnata

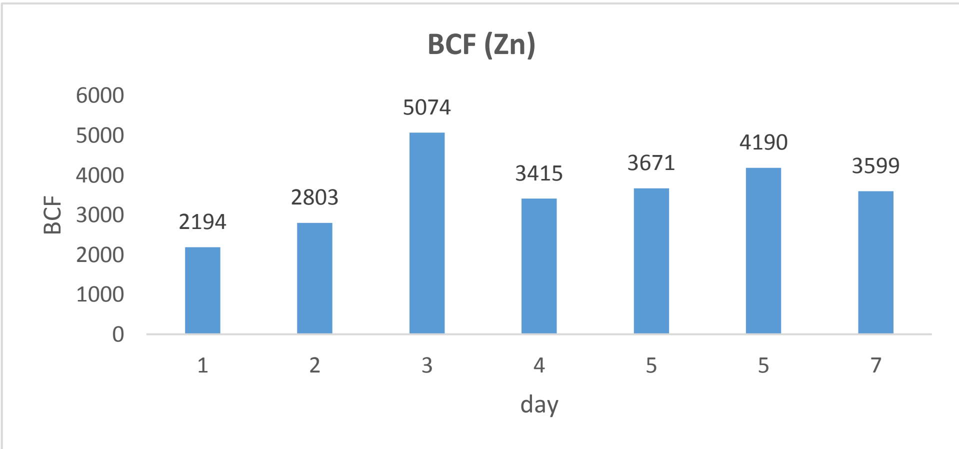


Fig. 13 : BCF A. pinnata of zinc

In this study, the zinc amounts in the biomass *A. pinnata* on the first day is around 130.0 mg.kg^{-1}, that concentration is used as reference during the experiment study *(Fig. 12)*. The importance of zinc in the plant has long been demonstrated by the work of Mazé in 1914, cited by Zhang, (2002). Its major role in the plants is linked with auxin. It has an important physiologic role particularly important in three domains: polynucleotide synthesis and protein, metalloenzymes and carbohydrate metabolism (Behrouz, 1995). So, Zn is vital for higher plants and is involved in some metabolic processes (Paschke et *al.*, 2000; Price et *al.*, 1972).

After the first day of experiment study *A. pinnata* accumulated 295.1 mg Zn.kg^{-1}, the plant performance to accumulate the zinc is 127.0% then. It accumulated more than 2200 times greater than zinc in its biomass, knowing that the rate of that metal in the wastewater is around 132.55 µg.L^{-1} (*table 2*). The variation is growing between the first and the third day of experiment study. Also, on the third day, the plant performance is the maximum, it can reach until 417.1% and in that case the BCF is 5074 (*Fig.13*). *A. pinnata* can accumulate until 5170 times more than zinc in its tissue compared to the quantity found in the polluted site. On the fourth day of experience, contrariwise, the plant capacity to accumulate zinc decreased until 243.1%. That is because of saturation by the copper of the plant (Merve et *al.*, 2015). On the fifth day, we find an increase of 270.2%, then for the sixth and the last day, that performance declines and becomes 264.5%. The zinc amounts accumulated in the *A pinnata* (DW) during those 7 days of treatment are: 295.1 mg.kg^{-1} the first day, 374.8 mg.kg^{-1} the second day and 672.3 mg.kg^{-1}, 446.1 mg.kg^{-1}, 481.3 mg.kg^{-1}, 556.9 mg.kg^{-1} and 469.9 mg.kg^{-1} the following days.

Zinc has a strong linear correlation with manganese and chromium. The Pearson's correlation coefficients are respectively 0.7068 (Zn-Mn) and 0.8038 (Zn-Cr). Merve *et al.,* (2015), it was found in their experience that the Zn is strongly correlated to Fe, Pb and As. That metal, contrariwise, does not have any negative correlation with other metals. The correlation coefficients of Spearman between Zn-Fe, Zn-Pb, and Zn-As are respectively 0.76; 0.81 and 0.86 (Merve et *al.,* 2015).

4. SUMMARY

Compared to other metals, the chrome (Cr), Copper (Cu), manganese (Mn) and zinc (Zinc) content are predominant in surface water, but only manganese (Mn) and zinc (Zn) dominate in the well water used by the citizen of Andralanitra. The rates of those metals are respectively 103.36 µg Zn.L^{-1} and 9487.83 µg Mn.L^{-1} in the well water (*table 1*) and 70.86 µg Cr.L^{-1}; 121.52 µg Cu.L^{-1}; 265.71 µg Mn.L^{-1} and 132.55 µg Zn.L^{-1} (*table 2*) for the surface water. The increase uptake of those metals by *A. pinnata* versus time is not linear, that is firstly due to the variation of physics parameter of the growing environment together with the climate condition and demographic of the site; and secondly, the existence of competition and interaction between ions of the nutrient medium (Falihery et *al.,* 2020). Many experiences showed the synergy or the antagonism between heavy metals and other elements. Coïc and Coppenet, (1989), marked the antagonism between P-Zn; Page and *al.,* (1981); Sikora and Wolt, (1986) and McKenna and *al.,* (1991), the antagonism between Cd-Zn and Burton et *al.,* (1985) the antagonism between Cd-Cu. However, the increased absorption of Cd, by the plants is observed in the presence of chlorides (Bigham et *al.,* 1984). During our experimental study, we find that *A. pinnata* has potentiality to accumulate metals Cr, Cu, Mn and Zn in its biomass. However, the maximum amount accumulated together with the accumulation is different. *A. pinnata* eliminates 1167.9 mg.kg^{-1} of Mn > 683.1 mg.kg^{-1} of copper > 672.3 mg.kg^{-1} of zinc > 151.8 mg.kg^{-1} of Cr during the 7 days of cultivation. Concerning the BCF, that with Cu is the greatest, followed by that of Zn, then that of Mn and that of Cr is the smallest, so BCF (Cu = 5465) > BCF (Zn = 5074) > BCF (Mn = 4368) > BCF (Cr = 2275). The high value of BCF indicates the plant capacity to accumulate pollutants in its tissue together with its potentiality on the phytoremediation (Zhang *et al.,* 2002). Those results show us that the floating plant *Azolla pinnata* is one of the aquatic plants which have a high potentiality to treat metals Cr, Cu, Mn and Zn and it is used in the technology of phytoremediation due to that. Also, *A. pinnata has been* used in different polluted site to treat Hg and Cd metals (Rai, 2008). Compared to other macrophytes spices, duckweeds show an interesting accumulation potential. Compared to *Typha latifolia* (Cattail), *Fimbristylis dichotoma* (Rush) and *Eichhornia crassipes*, *Azolla* has a superior performance to accumulate metals, it is more efficient during the heavy metals treatment (correlation and Jaiswal, 2008) such as Cu, Zn and Mn. Well, the obtained BCF *A. pinnata* are superior to those observed at *Typha spp.* and *Spartina spp.* In the phytoaccumulation technique, *Azolla* (macrophytes) is very effective in treating heavy metals; it has an excellent capacity in its use phytoremediation (Maiti and Jaiswal, 2008).

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DATA SUPPLEMENT

Table 1 : Physicochemical characteristics of the Well water

	CE mS.cm^{-1}	T°C	pH	(µg.L^{-1})				(Mg.L^{-1})				
				Cu	Zn	Co	Mn	HCO_3^-	NO_3^-	SO_4^{2-}	Mg	Ca
j1	3,26	24	5,82	27,12	103,7	39,7	9479,4	128,1	23,15	118,05	68,04	48
j2	3,17	25,5	5,61	25,31	105,48	41,85	9532,5	130,7	22,58	120,11	65,37	47,54
j3	3,1	25	6,21	28,42	100,91	38,72	9451,6	125,4	24,32	125,62	64,28	47,32
Moyenne	**3,18**	**24,83**	**5,88**	**26,95**	**103,36**	**40,09**	**9487,83**	**128,07**	**23,35**	**121,26**	**65,90**	**47,62**
Dev. Std	**0,08**	**0,76**	**0,30**	**1,56**	**2,30**	**1,60**	**41,10**	**2,65**	**0,89**	**3,91**	**1,93**	**0,35**

Table 2 : Physicochemical characteristics of the Wastewater

	pH	CE mS.cm^{-1}	T°C	µg.L^{-1}					mg.L^{-1}					
				Mn	Cu	Zn	Cr	Al	HCO_3^-	NO_3^-	Ca	Mg	Na	Fet
j0	6,17	25,6	26,5	265,4	123,9	134,4	67,2	137,9	9,51	0,97	24,58	42,17	7,43	3
j1	6,32	24,3	25,5	265,5	120,31	134,5	63,54	135,91	9,52	0,98	24,55	42,18	7,43	2,95
j2	7,1	24,12	26	265,3	120,98	133,72	72,12	132,49	9,61	0,97	24,5	42,06	7,41	2,8
j3	7,54	25,32	27	267,1	120,05	132,51	74,41	133,96	9,6	0,97	24,41	42,16	7,4	2,78
j4	7,1	26,12	26,5	266,4	119,36	130,64	66,72	131,66	9,58	0,96	24,3	42,15	7,38	3,05
j5	6,41	25,12	27	264,3	118,41	131,11	67,31	135,42	9,5	0,98	24,47	42,16	7,22	3,1
j6	6,32	24,21	28	265,6	124,11	132,91	77,79	137,11	9,47	0,95	24,5	42,17	7,41	3,02
j7	7,21	24,31	26	266	125	130,58	77,82	136,67	9,48	0,97	24,52	42,08	7,43	3,02
Moyenne	**6,77**	**24,89**	**26,56**	**265,70**	**121,52**	**132,55**	**70,86**	**135,14**	**9,53**	**0,97**	**24,48**	**42,14**	**7,39**	**2,97**
Dev. Std.	**0,52**	**0,76**	**0,78**	**0,83**	**2,47**	**1,62**	**5,44**	**2,24**	**0,06**	**0,01**	**0,09**	**0,05**	**0,07**	**0,12**