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REMOVING TOXIC PB^{+2} IONS FROM WATER USING BIOMONITOR MACROALGAE, PLANTS, AND NANOMAGNETITE PARTICLES

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Water pollution, lead, biosorption, adsorption, nanomagnetite, biomonitor, macroalgae, pine resin, moss

ABSTRACT
In our project, we researched the treatment of wastewater and the sea from toxic lead (Pb^{+2}) ions by thoke biosorption ability of biomonitor organisms and the adsorption capabilities of biocompatible nanomagnetite particles. Magnetite particles, macroalgae, and plants that have high adsorption capabilities were investigated to see if they can be an alternative to active carbon, the substance used at the lead treatment in water that has a high production cost. The protection of water and its quality is really important for keeping ecological balance and for organisms to sustain their lives. However, due to climate change, environmental damages and pollution, a huge part of the world is experiencing a water shortage. This is why water pollution has become one of the most major environmental problems of the century. Toxic heavy metals are on of the factors that contribute heavilty to water pollution. Moreover, the lead pollution in the seas can reach us, humans, through the food chain and can cause serious health problems like cancer. Based on this, we intended to test nanomagnetites as a natural adsorbent and the biosorption ability of macroalgae and terrestrial plants in lead treatment. According to this, in the first phase of our method we collected water samples from the sea and sent them to lead analysis. Next, we synthesized the nanomagnetite particles in our laboratory and administered it to the synthetic lead solution we had prepared. In the following phase, we administered the natural adsorbents *Ulva lactuca, Codium fragile, Rhodophytes, Bryophytes,* and pine resin again to the synthetic lead solution. We applied the same procedure to active carbon. Lastly, we sent our samples to ICP-MS analysis for the analysis of the lead left in our solutions. According to our analysis results, the Pb^{+2} ion adsorption capacity is respectively 0.2 g nanomagnetite (%83), active carbon (%80), pine resin (%76), *Ulva lactuca* (%64), *Codium fragile* (%51), moss *(Bryophytes)* (%46) and 0.1 g nanomagnetite (%32). As a consequence, we proposed the research of natural and economic adsorbents for lead treatment and for these types of research to be given more importance.

Introduction

Water is essential for living organisms to sustain their life. While seventy percent of the world is covered in water, only %0.3 is usable. Thus the protection of such water sources and maintaining their qualities becomes highly important for all organisms. However, due to external factors such as climate change, damages to habitats, pollution etc. approximatly %40 of the world experiences water shortages. (ÇEKUD,2014) The rapid increase in the population of the world causes the need for water to increase proportionally, to which the current water suplies cannot compensate for. Furthermore; pollution has become an important environmental risk for the already limited water supply. Pollution can include many different factors yet the most important ones remain the toxic heavy metals.

Figure 1: Water pollution and purification

The metals in natural minerals are commonly found as insoluble substances and pose no harm to living organisms. On the other hand, the ones that are soluble show toxic properties. Pb, Cr, Cd, and Mn can be given as examples of heavy metals that show toxicity. With industry wastes and the fuel of sea vehicles mixing into water, lead can be detected in sea creatures and in humans' tissues through the food chain. There has to be an accumulation of lead in blood or soft tissues to an extent to have a toxic effect on the body. Its effect varies according to various factors like age, diet and physiological condition. In children, toxic effects are seen at 40-80 µg Pb/ 100 mL while in adults the limit for lead poisoning is 80 µg Pb/ 100 mL. The amount of lead in hair, bones, and teeth can inform us about the state of lead poisoning. Acute lead poisoning can cause brain damage and death, while in babies and children - if exposed at a young age - it can lead to mental deficiency, learning disorders and hyperactivity, high blood pressure, chronic anemia, and peripheral neuropathies. (Özbolat & Tuli, 2016)

In our country, some examples of the factors that cause lead pollution are: uncontrolled industrialization and urbanization, rapid population growth, and the use of pesticide and chemical fertilizer. It's a known fact that especially wastewater of industries causes environmental damages through water and soil pollution. The most important thing is that heavy metals can climb up through the food chain until the ultimate consumer, which is us, humans. This biological accumulation that can happen in human tissues can cause respiratory and circulation system problems and even cancer. Especially lead (Pb^{+2}) accumulates in the human body happens %65 through food, %20 through water, and %15 through the air. (Dündar & Aslan, 2005)

Today when water pollution is mentioned, the first thing to come to our minds is the pollution in seas. It is speculated that in the Marmara Sea, straits, and gulfs, the wastes containing heavy metals due to industry and sea freight is on the rise. The fuels of sea vehicles, which contain lead, can mix into the sea and are seen as an important factor of lead pollution. The most common method used today to determine the level of pollution in water is generally the chemical analysis of water. However, as an alternative, the algae from aquatic organisms can also be thought of as a pollution indicator. Because algae are in balance with the water environment they live in, they can represent the potential pollution level of water in biological terms. These kinds of organisms that can show the pollution levels through biological accumulation or other observable effects are called bioindicators or biomonitors (Garty 2001). Biomonitor organisms have the ability to collect toxic substances. More so, according to the change in the level of toxic substances in the environment, the amount of toxic substances they can collect can also change. (Tunca, 2012)

So, we decided to study biomonitor algae types from the coasts of the polluted Marmara Sea. *Ulva lactuca (sea lettuce), Codium fragile (green sea fingers),* and *Rhodophyta (red algae)* were seen the most at dirty coasts. From the hypothesis that these algae can play a role in heavy metal treatment, the first phase of our project was planned.

Figure 2: *Ulva lactuca*, *Rodophyte* and *Codium fragile*

Algae is actually one of the most important parts of the sea ecosystem and is used by humans in a lot of different things like: medicine, pharmaceutics, cosmetics, food, agriculture and industry. It is known that algae have been an important food source in Far East countries since the 17th century. While algae aren't used as a food source much in Western countries and the USA, they use it in biochemical and technological researches. As a result, in a lot of countries, an algae-reliant industry has formed (Aktar, S & Cebe, G.). To prevent algae scarcity in industry, algal culture is used alongside harvesting naturally growing algae from the sea. We, however, wanted to try algae in heavy metal treatment instead of their other properties. Of these algae, *Ulva lactuca* is a green alga that is seen worldwide. Commonly found in shallow waters, it can reproduce sexually or asexually (vegetative). It reproduces rapidly, spreading to cover the water surface. Another property of Ulva lactuca is its biosorption ability like other algae. Biosorption is the absorption of heavy metal by the biomass in the aqueous medium. Dissolved substances that are kept on the biosorbent surface must pass through the solvent liquid film surrounding the biomass. (Ciuca A. et al, 2017) (El-Sikily, A., 2007)

Red algae *(Rhodophyta)* forms a huge part of the sea algae. These macro-algae are generally filamentous and have a leaf-like form. Red algae are the most sophisticated group of algae. They show a wide distribution in the world's seas. They are considered a close relative with blue-green algae in terms of pigment structures and membrane types. Red algae can use shorter wavelengths of light for photosynthesis, thanks to their pigments and compared to other algae, can live in deeper parts of the sea (150-200 m). (Rhodophyte, 2017).

Codium fragile belongs to the dark green algae group and can grow up to 1 meter in length. It is formed of double cylindrical branches and has a finger-like appearance. It's spread over a wide area in both northern and southern hemispheres, including the Pacific and the Southeast Pacific. It can withstand temperatures down to -2°C and can be found in open shores and tide pools. It can stick to rocks, shells and other hard surfaces. (Codium fragile,2019)

In this project, we tested terrestrial biomonitor plants and their products as well. Moss *(Bryophyta)* is a terrestrial nonflowering plant that mostly reproduces in moist habitats. Some species can also live in dry areas but still need moisture for reproduction. They can be found in any part of the world, but they are more common in tropical, temperate, and polar regions. They have no vascular bundles (xylem and phloem) or roots (Bryophytes, 2017). Mosses can be counted as biomonitor organisms because they can absorb the pollutants that reach them with atmospheric precipitation, and thus provide long-term information about the atmospheric pollution of the region where they develop, not just instantaneous information like other analysis methods. (Czora& et al,2012) Furthermore, there is research being done about whether mosses can remove heavy metals from water using adsorption. (Burçin, 2014)

Pine trees can also be counted as a biomonitor organism. Huhn and his friends have examined the heavy metal accumulation between the 60-years-old yellow pine shells in the center of Germany and the yellow pine shells from forested regions (Huhn, 1995). Resin is a natural substance secreted by pine trees to protect themselves and is both antibacterial and biocompatible, posing no threat to human health. Due to these properties, we wonder if pine resin can play a role in heavy metal treatment. Studies related to this are being done. (Kugler, 2019) We also decided on pine resin as one of our experiment groups to investigate its role in treatment.

In the second stage of our project, we considered synthesizing and using nanoparticles, one of the most important research being done in heavy metal treatment in recent years. Nano-iron oxide is used in cancer treatment research due to posing no threat to human health and its magnetic property attracted our attention. Compared to other nanoparticles nano-iron oxide (nanomagnetite) has high biosafety, is easier to obtain, can be synthesized at a cheap cost and is biocompatible. (Onar, K.&et al) Using their magnetic properties, nanomagnetite can be made to adsorb heavy metals from the liquid. Because it's easier to remove nanoparticles, which have collected the heavy metals from the liquid using adsorption, by applying a magnetic field. This way the heavy metals would be

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removed from the water. Adsorption is the adhesion of dissolved particles in a fluid to a solid surface. (Fato, Li, Zhao, Qiu, & Long, 2019)

In conclusion, in this project we aimed to find a biocompatible, cheap, environment-friendly alternative to classical and costly methods -like chemical precipitation and active carbon- in the removal of toxic metal ions like lead from wastewaters. We searched for new solutions methods that are both economic and have higher purification percentages using our natural refining materials' biosorption and nanomagnetites' adsorption ability. It is an important research subject to find natural solutions to water pollution that our world and all living things are experiencing increasingly.

Method

Materials:

Pb^{+2} stock solution at %99 purity, magnetic stirrer, Erlenmeyer flask, pH probe, red alga *(Gracilaria tikvahiae*), sea lettuce *(Ulva lactuca)*, green alga *(Codium fragile)*, pine resin, moss *(Bryophyta)*, water samples taken from the Marmara Sea, active carbon, NaOH, $FeSO_4$, $FeCl_3$, Poly(acrylic acid), orbital shaker, lyophilizator, Pb^{+2} standard solution (MERCK), micropipette.

Figure 3: Materials used for nanomagnetite synthesis

Preparation of Soltuions:

Preparation of Fe(II) solution: 0.242 g of $FeSO_4.7H_2O$ was weighed and dissolved in distilled water, then completed to 10 mL in a volumetric flask.
Preparation of Fe(III) solution: 0.282 g of $FeCl_3$ was weighed and dissolved in distilled water, then completed to 10 mL in a volumetric flask.
Preparation of basic solution: 0.514 g of NaOH was weighed and dissolved in distilled water, then completed to 20 mL in a volumetric flask.
Preparation of Poly(acrylic acid) solution: 0.188 g of poly(acrylic acid) was weighed and dissolved in distilled water, then completed to 80 mL in a volumetric flask.
Preparation of Synthetic Pb^{+2} solution: 0.1 mL Pb^{+2} was added to 1000 mL distilled water.

Figure 4: Preparation of synthetic 0.1ml/L Pb^{+2} wasetwater sample

Synthesis of Magnetite Nanoparticles and Their Application to Synthetic Pb^{+2} Solution:

The co-precipitation method, which is used frequently in literature, was used because it's an easy method for the synthesis of magnetite particles and has high efficiency. In this method, first 50 mL distilled water was poured into a jacketed glass reactor that had been heated to 60°C, using a circulator pump. Then nitrogen gas was applied for 30 minutes to cleanse the reactor from oxygen to prevent iron oxidization during the synthesis. Afterward, the prepared poly(acrylic acid) solution was added to 50 mL of water. Then, $FeSO_4$ and $FeCl_3$ solutions were added to the jacketed glass reactor, respectively. After another 30 minutes of mixing, the basic solution was added to the reaction. After this addition, it was observed that the color of the solution suddenly changed from dark yellow to black. With this color change, it was understood that magnetite particles were formed.

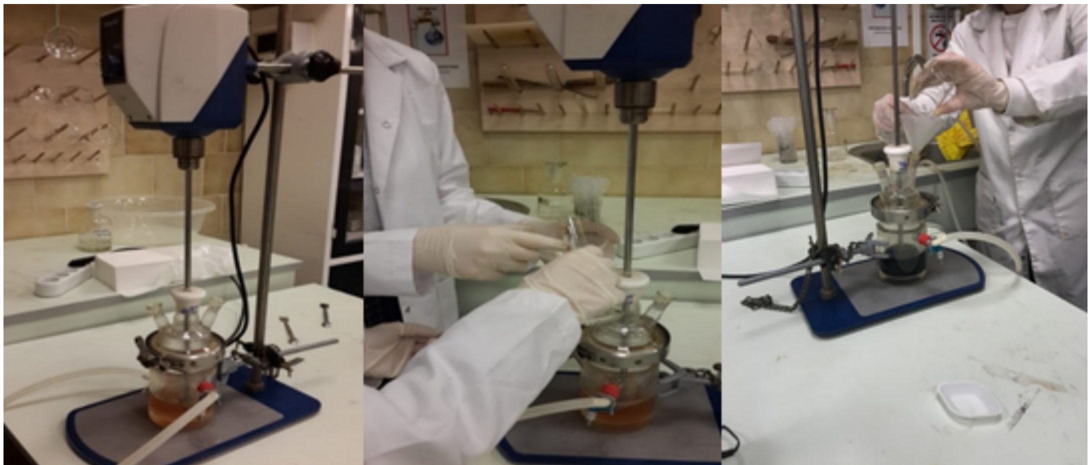

Figure 5: Adding $FeSO_4.7H_2O$ and $FeCl_3$ solutions to the jacketed glass reactor, the abrupt color change from dark yellow to black when the base solution (NaOH) is added.

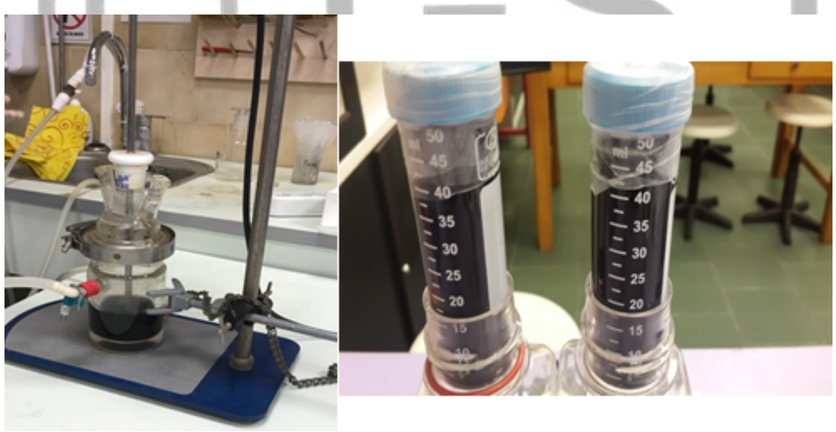

Figure 6: Solution containing nanomagnetite particles

After 30 minutes of mixing, the reaction suspension was taken from the reactor and the synthesized nanoparticles were collected by a magnet. To ensure no residual remains on the collected particles, they were washed twice with distilled water and centrifuged at 6000 rpm for 15 minutes. Then the magnetite nanoparticles were placed in lyophilizator to solidify them, and after 1-day magnetite nanoparticles in powder form were obtained. After taking 50 ml from the 0.1ml/L Pb^{+2} solution, 0.1 g and 0.2 g of powdered magnetite nanoparticles were added. Then it was placed in the orbital shaker and mixed at 25°C at 100 rpm for 5 hours. At the end of mixing, the samples were removed from the orbital shaker and the magnetite nanoparticles were removed from the medium with the use of a magnet. The adsorbent free samples were sent to ICP-MS (Inductively coupled plasma mass spectrometry) analysis for heavy metal (Pb^{+2}) quantification.

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Application of Natural Adsorbents and Active Carbon to Synthetic Pb^{+2} Solution:

The alga samples were collected from the Marmara Sea. The plant samples and the pine resin were collected from the forest area in Yalova province. The active carbon was supplied from a chemistry lab.

Figure 12: Algae in the Marmara sea

Figure 13: Collecting the algae from the shore

Figure 13: Other adsorbents used in Pb^{+2} treatment

The pH of the prepared 0.1ml/L Pb^{+2} solution was measured using a pH probe and a pH meter. The pH was determined to be 4.81.

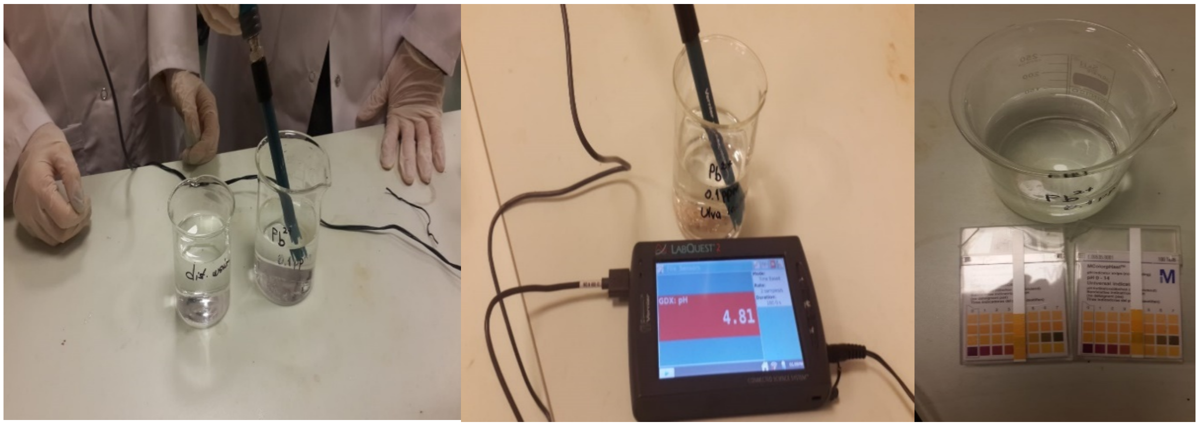


Figure 14: Measuring pH of 0,1ml / L Pb^{+2} solution with pH sensor and pH meter

Then, 100ml of 0.1ml/L Pb^{+2} solution was taken and placed in 7 different 150ml beakers. The samples were weighed on a precision scale to be 5 g and were placed in the beakers. Then the beakers were placed on the magnetic stirrer and rotated at 100 rpm at 25°C for 5 hours.

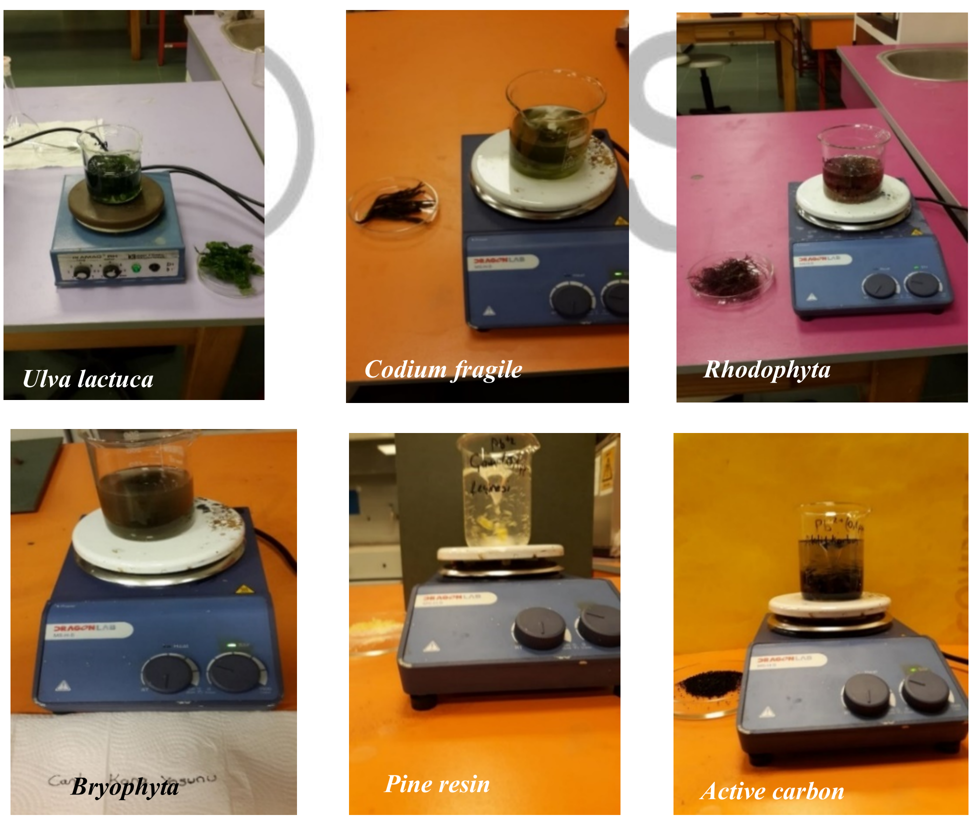


Figure 15: Stirring the samples with 0,1 ml / L Pb^{+2} solution for 5 hours with a magnetic stirrer.

After 5 hours, the solutions were filtered twice with filter paper.

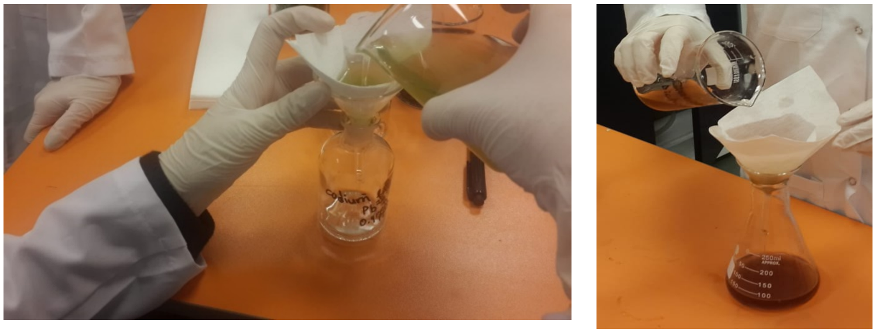


Figure 16: Filtration of samples after 5 hours

After the filtration, all the adsorbent free samples were sent to ICP-MS (Inductively coupled plasma mass spectrometry) analysis for heavy metal (Pb^{+2}) quantification.

Figure 17: All samples to be sent to ICP-MS analysis

Seawater samples taken from the shores of the Marmara Sea were also sent to the same location for heavy metal (Pb^{+2}) analysis.

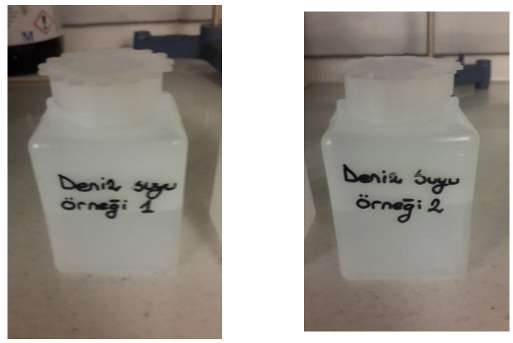


Figure 18: Seawater samples taken from two different shores of the Marmara Sea

Results

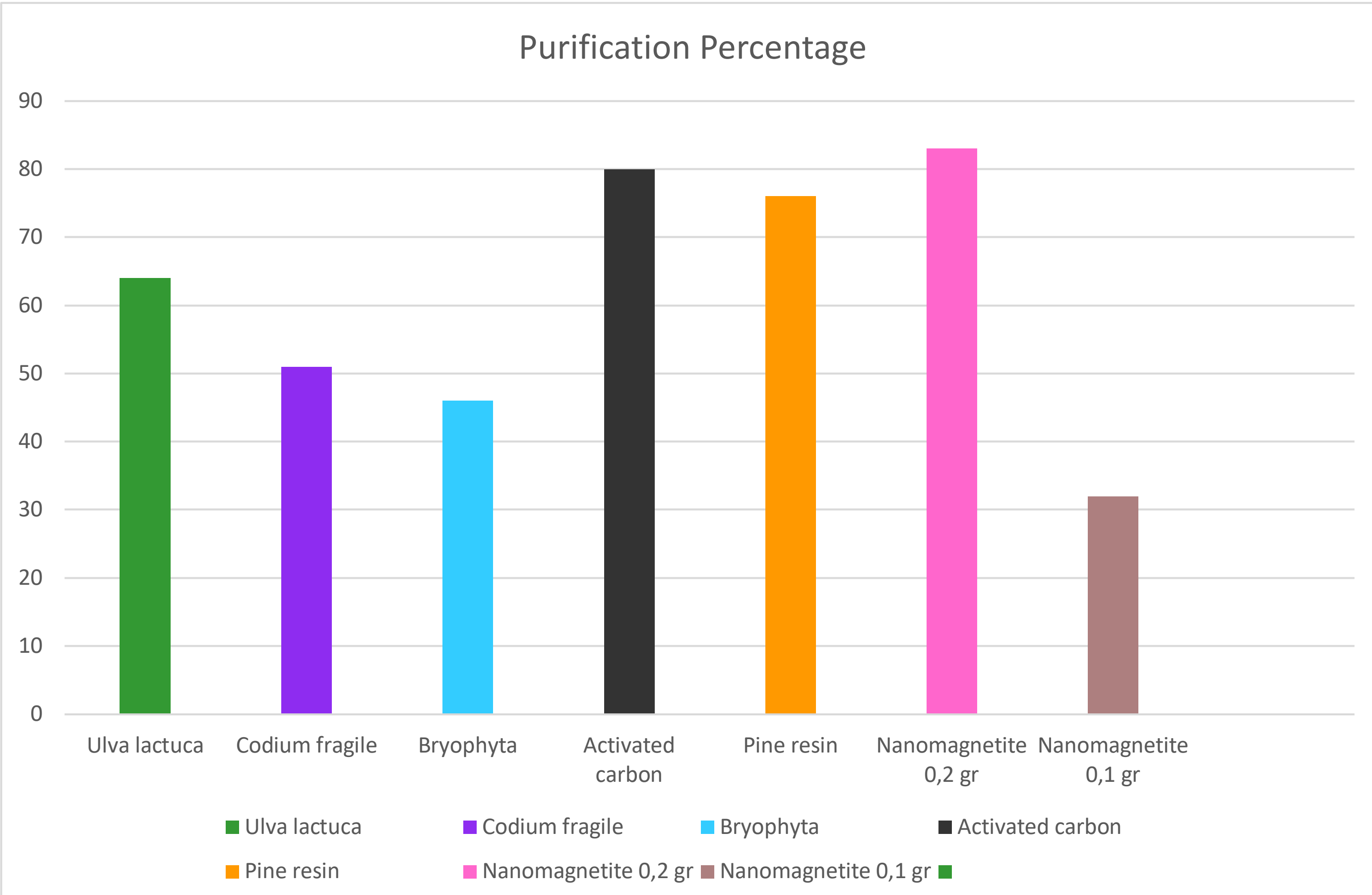
Table 1: ICP-MS Analysis Results

Type and amount of adsorbent	*After adsorption Pb^{+2} mg/L*
Control group (0,1mg/L Pb^{+2} Solution that has no adsorbent)	0.088
Ulva lactuca(5g)	0.032
Codium fragile(5g)	0.043
Rhodophyta (5g)	0.086
Bryophyta(5g)	0.047
Active carbon *(5g)*	0.018
Pine resin powder*(5g)*	0.021
Nanomagnetite (0,2 g)	0.015
Nanomagnetite (0,1 g)	0.06

Table 2: Amount of Pb^{+2} Purification

Type and amount of adsorbent	*Amount of purification* (%)
Ulva lactuca(5g)	64
Codium fragile(5g)	51
Rhodophyta (5g)	Not purified
Bryophyta(5g)	46
Active carbon *(5g)*	80
Pine resin powder*(5g)*	76

Nanomagnetite (0,2 g)	83
Nanomagnetite (0,1 g)	32



Graph 1: Samples 0.1 ml/Pb^{+2} purification amount

Table 3: Lead in water samples taken from different regions of the Marmara Sea Pb^{+2} determination

Sea water samples	**Amount of Pb^{+2} (mg/L)**
Sea water from the clean area (1L)	**<0,01**
Sea water from the dirty area (1L)	**7,304**

Conclusion

In this project, biomonitor algae and terrestrial plants and their products -which reduce the toxic lead amount in wastewaters and sea with biosorption method- were used due to their reduced costs, ability to reproduce rapidly, and purification ability. In addition, the effect of biocompatible natural nanomagnetites on the treatment of Pb^{+2} ion by the adsorption method was investigated. The data obtained were compared with the chemical adsorbent active carbon.

Firstly, if we take a look at the lead content of the samples taken from a coastal region and a pier area, we can see that pier area's lead concentration was quite high. The reason for this was thought to be sea vehicles' fuel plus industrial wastes. From this point of view, it was concluded that some areas may have serious water pollution.

According to the results of our analysis, the adsorption capacity of our samples for Pb^{+2} ion is: 0,2 g nanomagnetite (%83), active carbon (%80), pine resin (%76), *Ulva lactuca* (%64), *Codium fragile* (%51), moss *(Bryophytes)* (%46) and 0.1 g nanomagnetite (%32), respectively. It was understood that red algae *(Rhodophytes*) did not purify.

Chemical active carbon is the most used adsorbent today in the refining industry. It also got our attention in our project with its %80 purification efficiency. However, it was seen that the purification percentage of nanomagnetite particles, which have been used in cancer studies in recent years, is higher than active carbon. Magnetite nanoparticles were synthesized by the co-precipitation method frequently encountered in the literature. Cations and anions in wastewater were removed from the environment by functionalizing hydrophilic and hydrophobic surfactants and polymers on their surfaces. Therefore, it was thought to cause high purification efficiency. Because a small amount of nanomagnetite is used, it's not chemical and easy to produce in short time, it was thought to be an adsorbent that can be proposed for use in lead ion treatment. Also, our results enabled us to conclude that increasing the amount of nanomagnetite adsorbent increases the adsorption capacity. Considering the high adsorption properties of nanomagnetites synthesized in laboratory conditions, we believe that it can be an alternative to high-cost active carbon in the removal of impurities from wastewaters.

It was a matter of curiosity whether natural pine resin, which is used in industry and health sector, would be effective in treatment. Considering the purification percentages, it's percentage of adsorption being very close to activate carbon's was evaluated as a positive result. Thus, pine resin was considered for recommendation to the treatment sector because it doesn't undergo any chemical process, it's not dangerous to health and has antibacterial properties.

It is thought that the pollution precursor biomonitor *Ulva lactuca* and *Codium fragile*, which can live in polluted areas, better adsorbed Pb^{+2} in wastewater by using electrostatic attraction force compared to other algae because their surfaces are more negatively charged. Moss, another biomonitor organism, adsorbed close to %50 of the lead which was considered a good result.

Thus, unwanted impurities in our seas and industrial wastewater can be economically treated thanks to the natural adsorbents we recommended and any damage to the ecosystem will be prevented. In addition, by applying simple methods to the adsorbent wastes, the impurities on their surfaces can be removed from the environment and made available again.

Since there's no production cost and chemical treatment, we recommend that feasibility studies are conducted of the natural adsorbents, primarily pine resin, *Ulva lactuca* and *Codium fragile* to use for treatment of Pb^{+2} ions from wastewaters and seas in pilot units. The fact is that active carbon, which is most preferred in the treatment of heavy metals, is both expensive and disposed, increasing the treatment cost considerably. However, the nanomagnetite particles that we synthesized, and consider in our project as an alternative to active carbon, has attracted attention by being both biocompatible and having a high purification rate. The high adsorption rate of small amounts of nanomagnetites is important for the country's economy. In addition, by creating magnetic fields using strong magnets, adsorbents can be removed from the water in a way that won't leave any residue. Therefore, nanomagnetites are recommended as reusable natural adsorbents in treatment plants. It should be remembered that toxic substances coming to the last trophic level of the food chain in the ecosystems will cause serious diseases, and it is recommended to raise the awareness of societies about the importance of water and to protect the waters of world against pollution. We recommend researching natural and economic adsorbents in water treatment and giving importance to these studies.In addition, using the treated water in areas such as irrigation in agriculture and environmental cleaning.

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