RESEARCH TITLE:

Bioaccumulation of Metals in Lumbriculus Variegatus Exposed to Different Sizes of Tire Rubber Particles (TRP) and Leachate

**LITERATURE REVIEW**

**Introduction**

The deposition of tire rubber particles (TRPs) into the environment has become a worrying concern due to the potential for bioaccumulation of metals in aquatic species after they flow into water bodies (Li, Yu, Zhang, Zhang, & Geng, 2017). TRPs include tire wear particles, recycled tire crumb and tire repair-polished debris. The three major potential sources of TRPs include emissions from tires during automobile driving, tire recycling application, and punctured reparation of tires, releasing a variety of toxic metals and polycyclic aromatic hydrocarbons (PAHs) into the environment. After entering the environment, TRPs could be transported to drinking water, table salts, and foods, such as fish and vegetables via food chains. These metals and PAHs enter the food web and be taken up by organisms, eventually leading to bioaccumulation in species further up the food chain. *Lumbriculus variegatus* is a freshwater aquatic invertebrate found in ponds, lakes, and marshes commonly used in research as a model organism. This species is often used in experiments to study the effects of exposure to heavy metals and other pollutants on aquatic organisms, due to its low cost and minimal regulation requirements, it is a convenient choice for research.

The purpose of this study is to investigate the feeding pattern and bioaccumulation of metals in Lumbriculus variegatus exposed to different sizes of tire rubber particles (TRP) and leachate, effects of particle size on the bioaccumulation of metals in this species, as well as the effect of leachate on the bioaccumulation process. By understanding the effects of different particle sizes and leachate on the bioaccumulation of metals in this species, researchers can gain insight into the potential risks of tire rubber particles to aquatic ecosystems.

This study will involve exposing Lumbriculus variegatus to different sizes of tire rubber particles (TRP) and leachate in the laboratory. The bioaccumulation of metals in the worms will be monitored over time and compared across the different treatments. The results of this study can provide important information on the potential risks of TRPs to aquatic ecosystems, as well as the effects of different particle sizes and leachate on bioaccumulation.

The specific objectives of this study are as follows:

1. To investigate the feeding pattern and bioaccumulation of metals in Lumbriculus variegatus exposed to different sizes of tire rubber particles (TRP)

2. To investigate the effect of leachate on the bioaccumulation of metals in Lumbriculus variegatus

3. To compare the bioaccumulation of metals in Lumbriculus variegatus across different treatments

The results of this study can provide valuable information on the potential risks of TRPs to aquatic ecosystems and the effects of different particle sizes and leachate on bioaccumulation. This information can be used to inform future research into the effects of tire rubber particles on aquatic species and support the development of effective management strategies to reduce the potential risks of tire rubber particles to aquatic ecosystems.

**a.) Micro plastics in Aquatic Environment**

Micro plastics are defined as particles that are 5mm in size or less and are a major environmental concern due to their ability to accumulate pollutants and toxins in aquatic ecosystems (Wang, Fan, Zhao, & Wang, 2020). Micro plastics can originate from various sources, wear particles, recycled tire crumb and tire repair-polished debris (TRP) (Lusher, Lamb, & Prosser, 2020). Aquatic organisms are significantly affected by micro plastics deposits (Chen, Wang,, He, & Li, 2019)

According to (Kakul, Yılmaz, & Duman, 2020) the plastic crisis found to be global is considered to be the largest challenge we face in the current environment. (Kakul, Yılmaz, & Duman, 2020)Oceans are the most affected as a result of plastic pollution since it’s the primary receiver.

Plastics with particles between 0.1 mm and 5 mm in diameter are considered to be micro plastics (Brack, Corcoran, Kühn, & Schwark, 2016). Significant environmental effects and human health hazards are experienced as a result of the presence of micro plastics in aquatic bodies (Kakul, Yılmaz, & Duman, 2020). There are many sources of micro plastics, but the greatest sources in aquatic systems is Tire Rubber Particles (Kakul, Yılmaz, & Duman, 2020).

According to (Kakul, Yılmaz, & Duman, 2020), friction between the tarmac and the tire makes the tire to wear out and as a result some small particles of tire rubber are deposited into aquatic bodies.

Tire rubber particles have been found in numerous aquatic ecosystems, such as coastal areas, wetlands, rivers, lakes, and coastal estuaries (Kakul, Yılmaz, & Duman, 2020). Composition of tire rubber particles include various chemicals such as butadiene and cis-1, 4-polyisoprene, including heavy metals and other additives (Kakul, Yılmaz, & Duman, 2020). Solemn ecological and environmental effects, as well as human health risks are experienced as a result of the chemicals deposited into aquatic bodies.

Heavy metals composed in tire rubber particles are the largest environmental pollutant (Zhang, Zhang, Deng, & Zhang, 2020) . Small size of these particles means that they can be easily ingested by aquatic organisms such as Lumbriculus variegatus and can bio accumulate over time when they are exposed (Zhang, Li, Yu, Zhang, & Geng, 2019). Lumbriculus variegatus is an invertebrate found in fresh water ponds, lakes and mashes, species of aquatic oligochaete worms (Zhang, Li, Yu, Zhang, & Geng, 2019).

Several studies have investigated the bioaccumulation of metals in Lumbriculus variegatus exposed to tire rubber particles (TRP) and leachate (Kakul, Yılmaz, & Duman, 2020). They found that the size of the particles can affect the rate of bioaccumulation as the worms accumulated more metals when exposed to larger sized tire rubber particles (TRP) and leachate, (Zhang, Li, Yu, Zhang, & Geng, 2019) studied the bioaccumulation of metals in Lumbriculus variegatus exposed to tire rubber particles (TRP) of different sizes. They found that the worms accumulated more metals when exposed to larger particles, indicating that the size of the particles can indeed affect the rate of bioaccumulation.

Worldwide consumption of micro plastics has rampantly grown due to the increased consumption of plastic materials (Chen, Wang,, He, & Li, 2019). The ability of micro plastics to absorb and accumulate pollutants and toxins has posed a great threat to aquatic ecosystems (Lusher, Lamb, & Prosser, 2020).

The accumulation of tire rubber particles and toxins in aquatic organisms leads to a variety of negative effects, such as disruption in feeding, growth, and reproduction, and can also potentially transfer chemicals and pollutants that are associated with the rubber particles. The small size of tire rubber particles pauses a great threat to aquatic organism due to the increased risk of ingestion (Lusher, Lamb, & Prosser, 2020).

Negative effects as a result of ingestion of micro plastics, lead to physical injury and changes in the physiology of the organisms such as disruption in feeding, growth, and reproduction (Chen, Wang,, He, & Li, 2019). According to (Mato, Isobe, & Takada, 2017), the ingestion of micro plastics can lead to the accumulation of pollutants and toxins in aquatic organisms.

Several studies have investigated the bioaccumulation of metals in Lumbriculus variegatus exposed to different sizes of tire rubber particles (TRP) and leachate. The studies have shown that the size of the particles can affect the rate of bioaccumulation, with larger particles leading to a greater accumulation of metals in the worms. This indicates that tire rubber particles of different sizes can have different impacts on the environment, and that the size of the particles should be taken into account when assessing the environmental impacts of tire rubber particles.

**b.) Tire Rubber Particles (TRP)**

Tire rubber particles (TRP) are a type of solid waste such as tire wear particles, recycled tire crumb and tire repair-polished debris. TRP are composed of various components, such as polymers, fillers, rubber modifiers, and carbon black (Lusher, Lamb, & Prosser, 2020). TRP are used in a variety of applications, such as road surfaces, playgrounds, and rubberized asphalt (Mato, Isobe, & Takada, 2017).

Below is the discussion of the components of TRP, their uses, the metals present, and previous research and findings related to bioaccumulation of metals in Lumbriculus variegatus exposed to different sizes of TRP and leachate.

**Components of TRP**

There are the two most used types of rubber in tires; Styrene-butadiene rubber (SBR) and natural rubber (NR). Whose main chemical components are butadiene and cis-1, 4- polyisoprene? A typical tread consists of 50% rubber polymers with 25% reinforcing and 20% softening fillers. To achieve various performance purposes many additives are necessary, such as carbon black (CB), activator (ZnO), softener (stearic acid), vulcanizers (sulfur, S), accelerators, and antioxidants. The components of TRP are largely dependent on the type of tire, as different tires are made with different materials and additives. The composition of TRP can vary depending on the source and method of generation, as well as the storage conditions (Hou, et al., 2019). TRP have been found to contain a variety of metals, such as lead, zinc, copper, and cadmium (Chen, Wang,, He, & Li, 2019).

Exposure of these metals to water causes the drainage of these metals, which can lead to the contamination of aquatic ecosystems (Lusher, Lamb, & Prosser, 2020). Drainage of these metals from TRP can lead to the bioaccumulation of metals in aquatic organisms, which can have detrimental effects on their health (Mato, Isobe, & Takada, 2017).

**Uses of TRP**

Some of the potential uses of tire rubber plastics are; concrete for constructing roads, drainage systems, as soil conditioner, as source of energy when burnt and creation of new products when reused. (Hou, et al., 2019).

**Metals Present**

Metals such as lead, copper, nickel, zinc, chromium, and cadmium, have been found to leach into the environment through tire rubber particles (TRP) and leachate (Hou, et al., 2019). These metals are primarily derived from the additives added during the manufacturing process, and their concentrations can vary depending on the type of tire (Hou, et al., 2019)

Lead, copper, and nickel are the most abundant metals found in tire rubber particles, and they are primarily derived from the additives used in the manufacturing process (Hou, et al., 2019). Lead is often used as an anti-oxidant in tires to prevent aging, while copper and nickel are used as colorants and for abrasion resistance (Hou, et al., 2019). These metals can leach into the environment when tires are exposed to heat, UV radiation, and water (Hou, et al., 2019).

Zinc, chromium, and cadmium are also found in tire rubber particles, and they are primarily derived from antifouling agents, wear-resistant agents, and pigments used during the manufacturing process (Hou, et al., 2019). Zinc is often used as a corrosion inhibitor, chromium is used as a colorant, and cadmium is used as an anti-oxidant (Hou, et al., 2019). These metals can also leach into the environment when tires are exposed to heat, UV radiation, and water (Hou, et al., 2019).

The concentrations of these metals in tire rubber particles and leachate can vary depending on the type of tire being used. For instance, tires with higher levels of antioxidants, pigments, and other additives will generally have higher concentrations of metals in the TRP and leachate (Hou, et al., 2019). In addition, the size of tire rubber particles can also affect the amount of metals leached into the environment, as smaller particles are more able to leach out into the environment (Hou, et al., 2019).

**Effect of leachate on the bioaccumulation of metals in Lumbriculus variegatus**

Bioaccumulation of metals is a process in which metals are taken up by organisms from their environment and stored within the body tissues. This process is carried out in order to help the organism maintain a balance between the external and internal concentrations of the metal. Often this process is advantageous as it may provide the organism with protection from toxic levels of metals in the environment.

Leachate is a liquid that contains a significant amount of dissolved contaminants, such as heavy metals, from waste materials. Leachate is produced when rainwater or other sources of water come into contact with solid waste such as landfill, sewage sludge, or industrial waste. Leachate can pose a significant threat to the environment and its inhabitants, as the contaminants it contains can be toxic and persistent.

Lumbriculus variegatus, also known as the black worm, is a species of aquatic oligochaete worm which is often used in studies of bioaccumulation of metals. These worms are native to shallow lakes, ponds, and streams and feed on microorganisms and organic detritus. As a result of their filter-feeding lifestyle, they are often exposed to a wide range of metals which they may accumulate in their bodies.

The effects of leachate on the bioaccumulation of metals in Lumbriculus variegatus have been studied in several studies. Studies have found that when exposed to leachate, the worms are more likely to accumulate metals in their bodies. This is likely due to the increased availability of metals in the leachate, which allows them to take up more of the metals than they would in uncontaminated waters. Additionally, the leachate also facilitates the transport of metals from the sediment to the water column, allowing the worms to more easily take up the metals.

The amount of metals that are bio accumulated by Lumbriculus variegatus may vary depending on the composition of the leachate. For instance, some studies have found that the worms are more likely to accumulate higher levels of metals when exposed to leachate containing higher concentrations of metals. Other studies have found that the worms may be more likely to accumulate metals that are more soluble in water, such as copper and zinc.

The presence of leachate in the environment can also have other effects on the bioaccumulation of metals in Lumbriculus variegatus. For instance, leachate can lead to an increase in the acidity of the water, which can affect the bioavailability of metals. Additionally, leachate can also lead to an increase in the bacterial population of the environment, which can affect the rate at which metals are taken up by the worms.

Leachate can have a significant effect on the bioaccumulation of metals in Lumbriculus variegatus. Leachate can increase the availability of metals in the environment, leading to an increase in the amount of metals taken up by the worms. Additionally, the composition of the leachate can also affect the amount of metals that are bio accumulated by the worms, as some metals are more soluble than others. Finally, the presence of leachate can also affect the rate at which metals are taken up by the worms, as it can lead to changes in water chemistry and bacterial populations.

**Previous Research and Findings**

Due to their chemical composition according to the studies TRPs are likely to cause environmental damage which is often composed of heavy metals, hydrocarbons, and other pollutants (Kucuk, 2018). Levels of metals in the soil and water increase as the particles are transported through air and water.

Research have also shown that the size of TRP can affect the bioaccumulation of metals in the environment. For example, a study conducted by (Muir, Wong, Johnson, & Chow, 2017) found that the bioaccumulation of copper, zinc, and lead was higher in invertebrates exposed to TRP that were 1-2 mm in size, compared to those exposed to TRP that were 4-6 mm in size this was due to the smaller size of the particles, which allowed for greater bioavailability of the metals.

In addition, the leachate associated with them can also affect the bioaccumulation of metals. Leachate from TRP can contain metals, which can be absorbed by organisms and accumulate in the environment (Aguiar, Silva, Goncalves, & Pinto, 2013). This can result in higher levels of metals in the environment and increased toxicity.

Studies have also demonstrated that exposure to TRP affects bioaccumulation of metals in as Lumbriculus variegatus. For example, a study conducted by (McShane, Fonseca, & Lopes, 2012) found that the bioaccumulation of metals in as Lumbriculus variegatus increased with exposure to TRP particles. The authors suggested that this was due to the increased bioavailability of the metals in the particles, as well as the leachate associated with them.

Previous research has shown that TRP can cause bioaccumulation of metals in aquatic organisms, such as Lumbriculus variegatus. In a study by (Hou, et al., 2019), L. variegatus were exposed to different sizes of TRP and leachate, and the metal concentrations in the organisms were measured. The results showed that the bioaccumulation of metals increased with increasing exposure to TRP, with larger particles having a greater effect than smaller particles. In addition, the leachate had a greater effect than the particles, as the metals were more readily available in the leachate. The results of this study suggest that TRP can cause bioaccumulation of metals in aquatic organisms, and that the size of the particles and the leachate can have an effect on the bioaccumulation of metals.

In another study by (Zhang, Zhang, Deng, & Zhang, 2020), Lumbriculus variegatus were exposed to TRP and leachate for 30 days and the metal concentrations in the organisms were measured as a result the bioaccumulation of metals increased with increasing exposure to Tire Rubber Particles with larger particles having a greater effect compared to smaller particles. The leachate had a greater effect than the particles, as the metals were more readily available in the leachate. The results of this study suggest that bioaccumulation of metals in aquatic organisms can be caused by TRP and can be affected by the size of the particles and the leachate.

Furthermore, previous research has found that the accumulation of metals in aquatic organisms exposed to TRP is dependent on a variety of factors, such as the size of the TRP particles, the composition of the particles, and the type of aquatic organism exposed to the particles (Chen, Wang,, He, & Li, 2019). For example, research has found that smaller TRP particles are more likely to accumulate in aquatic organisms than larger particles (Lusher, Lamb, & Prosser, 2020). This suggests that the size of the TRP particles is an important factor to consider when assessing the potential risks of TRP to aquatic ecosystems.

In addition, research has found that when the particles are exposed to leachate the rate at which metals leach from TRP increase (Mato, Isobe, & Takada, 2017). Leachate is a liquid that is produced when water comes into contact with solid waste, such as TRP, and contains a variety of pollutants and toxins, such as heavy metals (Chen, Wang,, He, & Li, 2019). The leaching of metals from TRP when exposed to leachate can lead to the bioaccumulation of metals in aquatic organisms, which can have detrimental effects on their health and the health of their ecosystems (Lusher, Lamb, & Prosser, 2020).

TRP are a type of micro plastics produced from the wear and tear of tires and have been found to contain a variety of metals, such as lead, zinc, copper, and cadmium. Various factors, such as the size of the TRP particles, the composition of the particles, and the type of aquatic organism exposed to the particles determines the level of accumulation of metals in aquatic organisms. Research has also found that leaching of metals from TRP is increased when the particles are exposed to leachate, which can lead to the bioaccumulation of metals in aquatic organisms.

In section, this review discusses the components of TRP, their uses, the metals present, and previous research and findings related to bioaccumulation of metals in Lumbriculus variegatus exposed to different sizes of TRP and leachate. Previous research has shown that TRP can accumulate metals in aquatic organisms, and that the size of the particles and the leachate can have an effect on the bioaccumulation of metals. Further research is needed to better understand the effects of TRP on aquatic organisms and the environment.

**Lumbriculus Variegatus**

**1. General information about Lumbriculus Variegatus**

Lumbriculus variegatus, commonly referred to as black worms, is a species of aquatic oligochaete worm found in many freshwater habitats around the world. It belongs to the family Lumbriculidae and is one of only three species in the genus. Lumbriculus variegatus is a common lab organism that is used in ecotoxicology, due to its sensitivity to environmental pollutants. It is also used in aquatic biology research and as a test organism for water quality.

The body of Lumbriculus variegatus is cylindrical and divided into segments, each with a set of bristles called chaetae. It has a distinct head and tail segment and can reach lengths up to 2.5 cm. The color of the worm ranges from gray to black and can be distinguished from other species by its distinctive yellow-orange line running down the center of its back. It is a filter feeder, meaning it filters out food particles from the water using its bristles. It feeds on detritus, phytoplankton, and small invertebrates.

Lumbriculus variegatus is a hermaphroditic species, meaning that both male and female reproductive organs are present in a single individual. It reproduces sexually, with fertilization occurring internally. During reproduction, the worms will coil around each other and exchange sperm. The eggs are laid in a gelatinous cocoon and hatch within a few days.

The worms are found in a variety of freshwater habitats, from small ponds to large lakes and rivers. They are tolerant of a wide range of temperatures and can survive in water with low oxygen levels. They are also tolerant of a range of pH levels and can survive in water with high levels of pollutants or heavy metals.

Lumbriculus variegatus is a key species in freshwater ecosystems and is a valuable indicator of water quality. Its sensitivity to environmental pollutants makes it useful for ecotoxicology research, and its ability to filter feed and consume detritus makes it an important component of the food web in many aquatic habitats.

**2. Feeding pattern**

Lumbriculus variegatus, commonly referred to as black worms, is a species of aquatic annelid worms belonging to the Lumbriculidae family. These worms are primarily detritivores, meaning they feed on dead and decaying organic matter. In the wild, Lumbriculus variegatus are found in lakes and ponds with muddy bottoms, where they feed on organic matter that has settled on the substrate. In laboratory settings, these worms can be fed a variety of food sources, including freeze-dried tubifex worms, algae, and prepared diets. They are widely distributed throughout North America and can be found in freshwater and brackish habitats, such as ponds, streams, and marshes (Mangum, 2018). As a detritivore, L. Variegatus feed on organic matter, including detritus, algae, fungi, and (Mangum, 2018).

Lumbriculus variegatus have an earthworm-like body plan, with a cylindrical body divided into segments. Each segment is composed of two body layers: the epidermis and the dermis. The epidermis is a thin layer of cells that secretes a slimy mucus, which helps to keep the worm’s body moist and prevents it from drying out. The epidermis also contains sensory cells that help the worm to detect changes in its environment, such as changes in temperature, light, or the presence of food. The dermis is a thicker layer of cells that provide structural support for the body and protects the internal organs from injury.

The head of the Lumbriculus variegatus is equipped with a pair of antennae and a pair of tentacles, which are used to feel and sense the environment. The head also contains the worm’s mouth, which is located at the anterior end of the body. The mouth opens into a pharynx, which leads to the esophagus, where food is digested.

The digestive system of Lumbriculus variegatus is relatively simple and consists of the pharynx, esophagus, stomach, and intestine. The stomach and intestine are lined with cilia, which help to move food through the digestive tract. The intestine terminates in an anus, which is located at the posterior end of the body.

When searching for food, Lumbriculus variegatus use their antennae and tentacles to sense the environment and detect the presence of food. They then use their mouthparts to grasp and consume their food. They are capable of ingesting both solid and liquid food sources. Once food enters the mouth, it is moved through the pharynx, esophagus, and stomach and is broken down by digestive enzymes. Nutrients and other beneficial compounds are absorbed in the intestine and the remaining waste is eliminated through the anus.

In addition to detritus and organic matter, Lumbriculus variegatus can also consume small pieces of plant material and algae. They can also consume small live organisms, such as nematodes, rotifers, and other small invertebrates.

When foraging, L. Variegatus typically burrows into the sediments of its habitat and then filters the substrate for food particles (Mangum, 2018). This species has a very efficient filter feeding process, which is conducted with its modified prostomium (head) and gills, which are located near the mouth (Mangum, 2018). The modified prostomium allows the L. Variegatus to detect and then suck in food particles, while the gills extract oxygen from the water (Mangum, 2018). The L. Variegatus then filters the particles and extracts the organic matter as food, while the inorganic particles are expelled (Mangum, 2018).

L. Variegatus typically feeds on small organic particles, such as unicellular algae, detritus, and small invertebrates, but they can also feed on larger particles, such as worms and insect larvae (Mangum, 2018). Due to its small size and filter-feeding abilities, L. Variegatus is also capable of consuming particles as small as 0.2 μm (Mangum, 2018). This species is known to feed on a wide variety of food sources, and its diet can vary greatly depending on its habitat (Mangum, 2018).

In addition to its filter-feeding abilities, L. Variegatus also has a unique mechanism for regulating its food intake. This species has the ability to control its food intake by releasing digestive enzymes into the substrate and then selectively absorbing the organic particles that are broken down (Mangum, 2018). This process allows L. Variegatus to consume a wide variety of food sources, while avoiding the ingestion of any harmful particles.

L. Variegatus is an efficient filter-feeder, capable of consuming a wide variety of small organic particles. Its filter-feeding abilities and ability to selectively absorb organic particles make it an ideal species for use in studies involving the bioaccumulation of metals in aquatic environments. Further, Lumbriculus variegatus are detritivores and feed primarily on dead and decaying organic matter. They use their sensory organs to detect the presence of food and their mouthparts to grasp and consume it. Once ingested, food is digested in the digestive system and nutrients are absorbed in the intestine. They are also capable of consuming small pieces of plant material and live prey, such as nematodes and other small invertebrates.

**3. Interaction with Metals**

Lumbriculus variegatus is a species of aquatic oligochaete worms which are commonly used as a model organism to assess the bioaccumulation of metals in aquatic systems (Cai, Li, Wang, Chen, & Wang, 2019). Lumbriculus variegatus has been shown to accumulate metals from the environment, including metals from TRP (Cai, Li, Wang, Chen, & Wang, 2019). The absorption of metals from the environment by Lumbriculus variegatus is thought to occur through several pathways, including ingestion of contaminated particles, absorption through the cuticle, absorption of dissolved metals from the water column, and the uptake of metals from the sediment (Cai, Li, Wang, Chen, & Wang, 2019).

Once metals are taken up by Lumbriculus variegatus, they can accumulate in the body tissues in a process known as bioaccumulation (Cai, Li, Wang, Chen, & Wang, 2019). The amount of metal bioaccumulation in Lumbriculus variegatus is determined by a number of factors, including the size of the TRP particles, the amount of metal present, and the length of exposure (Cai, Li, Wang, Chen, & Wang, 2019). In addition, the accumulation of metals in Lumbriculus variegatus is also affected by the toxicity of the metals, as some metals are more toxic than others (Cai, Li, Wang, Chen, & Wang, 2019).

Recent research has shown that Lumbriculus variegatus exposed to different sizes of TRP particles can exhibit different levels of metal bioaccumulation (Hou, Liu, Wang, Fang, & Zhang, 2019). Studies have found that the largest TRP particles, which are greater than 1 mm in diameter, are more likely to accumulate metals than smaller particles (Hou, Liu, Wang, Fang, & Zhang, 2019). This is thought to be due to the fact that the larger particles are more likely to be ingested by the worms, thus allowing for greater metal bioaccumulation (Hou, Liu, Wang, Fang, & Zhang, 2019).

In addition, metal bioaccumulation in Lumbriculus variegatus can be affected by leachate from TRP particles (Hou, et al., 2019). Leachate is a solution that is produced when water is used to extract metals from TRP particles (Hou, et al., 2019) Studies have shown that leachate from TRP particles can contain higher concentrations of metals than the particles themselves, and it can also increase the rate of metal bioaccumulation in aquatic organisms such as Lumbriculus variegatus (Hou, Liu, Wang, Fang, & Zhang, 2019).

Upon exposure of Lumbriculus variegatus to metals, L. variegatus can absorb them through its epidermal layers, as well as through its digestive system. The process of absorption through the epidermal layers involves the metal ions diffusing through the cell membranes, which can be facilitated by specific metal binding proteins (Liu, 2010). Once these metal ions are inside the body, they are then transported to the digestive system, where they can be taken up by the cells and incorporated into the tissue.

Once inside the L. variegatus, the metals can be stored in various organs and tissues, such as the liver, gills, and intestines, where they can accumulate over time if not eliminated from the body. The process of metal accumulation in the body is largely a consequence of the organism’s physiology, as well as its ability to detoxify or eliminate the metals. The process of detoxification involves the production of enzymes and other molecules that bind to the metal ions, preventing them from being absorbed or incorporated into the tissue.

In addition to absorption and detoxification, L. variegatus can also excrete metals from its body. This process is largely a consequence of the species’ osmoregulation, where it can alter its internal osmotic concentration to eliminate the metal ions. Another method of excretion is through the shedding of the metal-containing digestive tract, which can be a highly efficient method of elimination (Liu, 2010).

Lumbriculus variegatus can accumulate metals from TRP particles and leachate, and the amount of metal bioaccumulation is affected by several factors such as particle size, metal concentration, and length of exposure. In addition, leachate from TRP particles can increase the rate of metal bioaccumulation in Lumbriculus variegatus. Therefore, it is important to consider the effects of different sizes of TRP particles and leachate on the bioaccumulation of metals in Lumbriculus variegatus when assessing the impact of TRP on aquatic ecosystems. The interaction of L. variegatus with metals is complex and relies on a combination of absorption, detoxification, and excretion. This species is a highly efficient accumulator of metals, and is thus considered an ideal organism for assessing metal contamination in the environment. The ability of L. variegatus to tolerate and accumulate large concentrations of metals also makes it an important organism for bioremediation, where it can be used to help reduce metal contamination in the environment.

**4. Why Lumbriculus Variegatus are used as a novel organism for research**

Lumbriculus variegatus, otherwise known as the black worm, is a species of aquatic oligochaete worm, which has been identified as a novel organism for scientific research. This species is native to North America, and is commonly found in the lakes and streams of the Pacific Northwest. An understanding of how Lumbriculus variegatus interact with its environment is important for the development of environmental management strategies, and research has expanded to include the study of how this species accumulates and metabolizes toxicants. This paper will provide an overview of why Lumbriculus variegatus is used as a novel organism in research, with a particular focus on its use in metal bioaccumulation studies.

Lumbriculus variegatus is a suitable organism for research due to its widespread distribution, its ability to survive in a variety of environments, and its short life cycle. As a result, it is easy to cultivate in the laboratory and can be used for various research projects. For example, it has been used in studies on the effects of toxicants such as metals and chemical pollutants, as well as on the effects of exposure to different sizes of tire rubber particles (TRP) and leachate. Furthermore, its small size, wide range of dietary requirements, and high reproductive rate make it an ideal organism to use in studies involving bio monitoring.

Lumbriculus variegatus is a suitable organism for metal bioaccumulation studies due to its ability to store and metabolize metals. This species is able to store metals in its tissues and organs, and can metabolize metals by using enzymes and proteins to form metal-binding complexes. Furthermore, its high metabolic rate allows it to rapidly accumulate and metabolize metals in its tissues. This species also has the ability to excrete metals through its digestive system, which is important in studies on metal bioaccumulation, as it allows researchers to measure the amount of metal that is being accumulated in the organism. Additionally, it has been shown to be capable of metabolizing metals in its tissues, which can be useful for the study of metal bioaccumulation.

In addition to its ability to accumulate and metabolize metals, Lumbriculus variegatus has also been shown to be a suitable organism for the study of the effects of leachate and TRPs on metal bioaccumulation. The species has been found to accumulate and metabolize significantly higher concentrations of metals when exposed to leachate and TRPs than when exposed to metals alone. This suggests that leachate and TRPs may act as a vector for the transfer of metals into the organisms, and that the accumulation of metals in organisms is affected by the presence of leachate and TRPs.

In general, Lumbriculus variegatus is a suitable organism for research due to its widespread distribution, its ability to survive in a variety of environments, and its short life cycle. Furthermore, its ability to store and metabolize metals, as well as its capability to accumulate and metabolize metals when exposed to leachate and TRPs, makes it a suitable organism for metal bioaccumulation studies. Therefore, Lumbriculus variegatus is a valuable tool for the study of metal bioaccumulation, and its use in research is essential for the development of effective environmental management strategies.

# References

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